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(54) Title: ANTI-BACTERIAL VACCINE COMPOSITIONS

(57) Abstract: Gram negative bacterial virulence genes are identified, thereby allowing the identification of novel anti-bacterial agents that target these virulence genes and their products, and the provision of novel gram negative bacterial mutants useful in vaccines.

ANTI-BACTERIAL VACCINE COMPOSITIONS

This application is a continuation-in-part of U.S. Patent Application Serial No: 09/545,199, filed April 6, 2000, which claims benefit of U.S. Provisional Patent Application Serial Nos. 60/153,453, filed September 10, 1999 and 60/128,689, filed April 9, 1999.

FIELD OF THE INVENTION

The present invention relates generally to the identification of genes responsible for virulence of *Pasteurellaceae* bacteria, thereby allowing for production of novel attenuated mutant strains useful in vaccines and identification of new anti-bacterial agents that target the virulence genes and their products.

BACKGROUND OF THE INVENTION

The family *Pasteurellaceae* encompasses several significant pathogens that infect a wide variety of animals. In addition to *P. multocida*, prominent members of the family include *Pasteurella (Mannheimia) haemolytica, Actinobacillus pleuropneumoniae* and *Haemophilus somnus. P. multocida* is a gram-negative, nonmotile coccobacillus which is found in the normal flora of many wild and domestic animals and is known to cause disease in numerous animal species worldwide [Biberstein, In M. Kilian, W. Frederickson, and E. L. Biberstein (ed.), *Haemophilus, Pasteurella, and Actinobacillus*. Academic Press, London, p. 61-73 (1981)]. The disease manifestations following infection include septicemias, bronchopneumonias, rhinitis, and wound infections [Reviewed in Shewen, *et al., In* C. L. Gyles and C. O. Thoen (ed.), <u>Pathogenesis of Bacterial Infections in Animals</u>. Iowa State University Press, Ames, p. 216-225 (1993), incorporated herein by reference].

Infection by *P. multocida* generally results from invasion during periods of stress, but transmission may also occur by aerosol or contact exposure, or via flea and tick vectors. In fowl, *P. multocida* infection gives rise to acute to peracute septicemia, particularly prevalent in domestic turkeys and wild waterfowl under stress conditions associated with overcrowding, laying, molting, or severe

climatic change. In cattle, a similar hemorrhagic septicemia follows infection and manifests conditions including high fever and depression, generally followed by quick death. Transmission is most likely through aerosol contact, but infection can also arise during periods of significant climatic change. In rabbits, infection gives rise to recurring purulent rhinitis, generally followed by conjunctivitis, otitis media, sinusitis, subcutaneous abscesses, and chronic bronchopneumonia. In severe infections, rabbit mortality arises from acute fibrinous bronchopneumonia, septicemia, or endotoxemia. Disease states normally arise during periods of stress. In pigs, common *P. multocida* disease states include atrophic rhinitis and bacterial pneumonia. Similar pneumonia conditions are also detected in dogs, cats, goats, and sheep. *P. multocida* is commonly detected in oral flora of many animals and is therefore a common contaminant in bite and scratch wounds.

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P. multocida strains are normally designated by capsular serogroup and somatic serotype. Five capsular serogroups (A, B, D, E, and F) and 16 somatic serotypes are distinguished by expression of characteristic heat-stable antigens. Most strains are host specific and rarely infect more than one or two animals. The existence of different serotypes presents a problem for vaccination because traditional killed whole cell bacteria normally provide only serotype-specific protection. However, it has been demonstrated that natural infection with one serotype can lead to immunological protection against multiple serotypes [Shewen, et al., In C. L. Gyles and C. O. Thoen (Ed.), Pathogenesis of Bacterial Infections in Animals. Iowa State University Press, Ames, p. 216-225 (1993)] and cross protection can also be stimulated by using inactivated bacteria grown in vivo [Rimler, et al., Am J Vet Res. 42:2117-2121 (1981)]. One live spontaneous mutant P. multocida strain has been utilized as a vaccine and has been shown to stimulate a strong immune response [Davis, Poultry Digest. 20:430-434 (1987), Schlink, et al., Avian Dis. 31(1):13-21 (1987)]. This attenuated strain, however, has been shown to revert to a virulent state or cause mortality if the vaccine recipient is stressed [Davis, Poultry Digest. 20:430-434 (1987), Schlink, et al., Avian Dis. 31(1):13-21 (1987)].

Another member of the Pasteurella family, A. pleuropneumoniae exhibits strict host specificity for swine and is the causative agent of highly contagious porcine pleuropneumonia. Infection normally arises in intensive breeding conditions, and is believed to occur by a direct mode of transmission. The disease is often fatal and, as a result, leads to severe economic loss in the swine producing industry. A. pleuropneumoniae infection may be chronic or acute, and infection is characterized by a hemorrhagic, necrotic bronchopneumonia with accompanying fibrinous pleuritis. To date, bacterial virulence has been attributed to structural proteins, including serotype-specific capsular polysaccharides, lipopolysaccharides, and surface proteins, as well as extracellular cytolytic toxins. Despite purification and, in some instances cloning, of these virulence factors, the exact role of these virulence factors in A. pleuropneumoniae infection is poorly understood.

Twelve serotypes of A. pleuropneumoniae have been identified based on antigenic differences in capsular polysaccharides and production of extracellular toxins. Serotypes 1, 5, and 7 are most relevant to A. pleuropneumoniae infection in the United States, while serotypes 1, 2, 5, 7, and 9 are predominant in Europe. There are at least three significant extracellular toxins of A. pleuropneumoniae that are members of the haemolysin family and are referred to as RTX toxins. RTX toxins are produced by many Gram negative bacteria, including E. coli, Proteus vulgarisa, and Pasteurella haemolytica, and the proteins generally share structural and functional characteristics. Toxins from the various serotypes differ, however, in host specificity, target cells, and biological activities.

The major A. pleuropneumoniae RTX toxins include ApxI, ApxII, and ApxIII. ApxI and ApxIII have haemolytic activity, with ApxI being more potent. ApxIII shows no haemolytic activity, but is cytotoxic for alveolar macrophages and neutrophils. Most A. pleuropneumoniae serotypes produce two of these three toxins. For example, serotypes 1, 5, 9, and 11 express ApxI and ApxII, and serotypes 2, 3, 4, 6, and 8 express ApxII and ApxIII. Serotype 10, however, produces only ApxI, and serotypes 7 and 12 express only ApxII. Those A. pleuropneumoniae serotypes that produce both ApxI and ApxII are the most virulent strains of the bacteria.

The Apx toxins were demonstrated to be virulence factors in murine models and swine infection using randomly mutated wild type bacteria [Tascon, et al., Mol. Microbiol. 14:207-216 (1994)]. Other A. pleuropneumoniae mutants have also been generated with targeted mutagenesis to inactivate the gene encoding the AopA outer membrane virulence protein [Mulks and Buysee, Gene 165:61-66 (1995)].

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At least eleven serotypes (1, 2, 5-9, 12-14 and 16) have been demonstrated within Mannheimia [Pasteurella] haemolytica [Angen, et al., Vet Microbiol 65(4):283-90 (1999)], a Pasteurellaceae species which is responsible for serious outbreaks of acute pneumonia in neonatal, weaned, growing and adult lambs, calves, and goats [Ackermann, et al., Microbes Infect 2(9):1079-88 (2000)]. Transportation, viral infections, overcrowding, and other stressful conditions predispose animals to M. haemolytica infection [Ackermann, et al., supra.] The leukotoxin (Lkt) of M. haemolytica is believed to play a significant role in pathogenesis, causing cell lysis and apoptosis that lead to the lung pathology characteristic of bovine shipping fever [Highlander, et al., Infect Immun 68(7):3916-22 (2000)] as well as lung injury in bovine pneumonic pasteurellosis [Jeyaseelan, et al., Microb Pathog 30(2):59-69 (2001)]. Lkt is a pore-forming exotoxin that has the unique property of inducing cytolysis only in ruminant leukocytes and platelets [Jeyaseelan, et al., (2001), supra.]. Cytolysis of many cell types is mediated by arachidonic acid (AA) and its generation by phospholipases is regulated by G-protein-coupled receptors [Jeyaseelan, et al., (2001) supra] Recent studies indicate that M. haemolytica Lkt binds to bovine CD18, the common subunit of all beta2 integrins [Jeyaseelan, et al., Infect Immun 68(1):72-9 (2000)]. It has also been shown that LFA-1 is a Lkt receptor, Lkt binding to LFA-1 is not target cell specific, Lkt binding to bovine LFA-1 correlates with calcium elevation and cytolysis, and bovine LFA-1 expression correlates with the magnitude of Lkt-induced target cell cytolysis [Jeyaseelan, et al., Infect Immun 68(1):72-9 (2000)].

In attempts to produce vaccine compositions, traditional killed whole cell bacteria have provided only serotype-specific protection [MacInnes and Smart, supra], however, it has been demonstrated that natural infection with a highly virulent

serotype can stimulate strong protective immunity against multiple serotypes [Nielsen, Nord Vet Med. 31:407-13 (1979), Nielsen, Nord Vet Med. 36:221-234 (1984), Nielsen, Can J Vet Res. 29:580-582 (1988), Nielsen, ACTA Vet Scand. 15:80-89 (1994)]. One defined live-attenuated vaccine strain producing an inactive form of the ApxII toxin has shown promise for cross protection in swine [Prideaux, et al., Infection & Immunity 67:1962-1966 (1999)], while other undefined live-attenuated mutants have also shown promise [Inzana, et al., Infect Immun. 61:1682-6, (1993), Paltineanu, et al., In International Pig Veterinary Society, 1992, p. 214, Utrera, et al., In International Pig Veterinary Society, 1992, p. 213].

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Because of the problems associated with vaccine formulations comprising bacterial strains with undefined, spontaneous mutations, there exists a need in the art for rational construction of live attenuated bacterial strains for use in vaccines that will safely stimulate protective immunity against homologous and heterologous *Pasteurellaceae* serotypes. There further exists a need to identify attenuated bacterial strains and genes required for bacterial virulence, thereby facilitating development of methods to identify anti-bacterial agents.

SUMMARY OF THE INVENTION

In general, the present invention provides materials and methods for production and use of vaccine compositions comprising attenuated gram negative bacteria. In one aspect, vaccine compositions of the invention comprise attenuated species in the *Pasteurellaceae* family of bacteria, which is known in the art and described, in part, in Dewhirst, et al., J. Bacteriol. 174:2002-2013 (1992), incorporated herein by reference in its entirety. Species in the family include, but are not limited to, A. actinomycetemcomitans, A. capsulatus, A. equuli, A. lignieresii, A. pleuropneumoniae (H. pleuropneumoniae), A. seminis, A. suis (H. suis), A. ureae (p. ureae), A. capsulatus, Bisgaard taxon 11, H. aegyptius, H. aphrophilus, H. aphrophilus (H. parainfluenzae), H. ducreyi, H. haemoglobinophilus, H. haemolyticus, H. influenzae, H. paracuniculus, H. paragallinarum, H. parahaemolyticus, H. parainfluenzae, (H. paraphrophilus), H.

paraphrohaemolyticus, H. paraphrophilus, H. parasuis, H. parasuis type 5, H. segnis, H. somnus, Haemophilus minor group, Haemophilus taxon C, P. aerogenes, P. anatis, P. avium (H. avium), P. canis, P. dagmatis, P. gallinarum, P. (Mannheimia) haemolytica, P. trehalosi (P. haemolytica biotype T), P. langaa, P. multocida, P. pneumotropica, P. stomatis, P. volantium (H. parainfluenzae), P. volantium, Pasteurella species A, Pasteurella species B, and Haemophilus paraphrohaemolyticus. Preferably, vaccine compositions comprise attenuated Pasteurella (Mannheimia) haemolytica, Actinobacillus pleuropneumoniae, Haemophilus somnus, or Pasteurella multocida bacteria. In a most preferred embodiment, vaccine compositions of the invention comprise attenuated Pasteurella multocida and A. plueropneumoniae bacterial strains.

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One aspect of the invention provides gram negative bacterial organisms containing a functional mutation in a gene sequence represented by any one of SEQ ID NOS: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174, or species homologs thereof, wherein the mutation inhibits or abolishes expression and/or biological activity of an encoded gene product (i.e., the polypeptide encoded by a gene); said functional mutation resulting in attenuated virulence of the bacterial strain. Functional mutations that modulate (i.e., increase or decrease) expression and/or biological activity of a gene product include insertions or deletions in the protein coding region of the gene itself or in sequences responsible for, or involved in, control of gene expression. Deletion mutants include those wherein all or part of a specific gene sequence is deleted. Also contemplated are compositions, and preferably vaccine compositions, comprising mutated and attenuated gram negative bacterial organisms, optionally comprising a suitable adjuvant and/or a pharmaceutically acceptable diluent or carrier. In order for a modified strain to be effective in a vaccine formulation, the attenuation must be significant enough to

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prevent the pathogen from evoking severe clinical symptoms, but also insignificant enough to allow limited replication and growth of the bacteria in the host.

The invention also provides polynucleotides encoding gene products that are required for virulence in gram negative bacteria. Polynucleotides of the invention include DNA, such as complementary DNA, genomic DNA including complementary or anti-sense DNA, and wholly or partially synthesized DNA; RNA, including sense and antisense strands; and peptide nucleic acids as described, for example in Corey, TIBTECH 15:224-229 (1997). Virulence gene polynucleotides of the invention include those set forth in SEQ ID NOs:1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174, or species homologs thereof, polynucleotides encoding a virulence gene product encoded by a polynucleotide of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174, or a species homolog thereof, and polynucleotide that hybridize, under moderately to highly stringent conditions, to the noncoding strand (or complement) of any one of the polynucleotides set out in SEO ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174, or species homologs thereof. The invention therefore comprehends gene sequences from Pasteurellaceae set out in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174, as well as related gene sequences from other

gram negative bacterial organisms, including naturally occurring (*i.e.*, species homologs) and artificially induced variants thereof. The invention also comprehends polynucleotides which encode polypeptides deduced from any one of the polynucleotides set out in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 164, 166, 168, 170, 172, and 174, and species homologs thereof. Knowledge of the sequence of a polynucleotide of the invention makes readily available every possible fragment of that polynucleotide. The invention therefore provides fragments of a polynucleotide of the invention.

The invention further embraces expression constructs comprising polynucleotides of the invention. Host cells transformed, transfected or electroporated with a polynucleotide of the invention are also contemplated. The invention provides methods to produce a polypeptide encoded by a polynucleotide of the invention comprising the steps of growing a host cell of the invention under conditions that permit, and preferably promote, expression of a gene product encoded by the polynucleotide, and isolating the gene product from the host cell or the medium of its growth.

Identification of polynucleotides of the invention makes available the encoded polypeptides. Polypeptides of the invention include full length and fragment, or truncated, proteins; variants thereof; fusion, or chimeric proteins; and analogs, including those wherein conservative amino acid substitutions have been introduced into wild-type polypeptides. Antibodies that specifically recognize polypeptides of the invention are also provided, and include monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, humanized antibodies, human antibodies, and complementary determining region (CDR)-grafted antibodies, as well as compounds that include CDR sequences which specifically recognize a polypeptide of the invention. The invention also provides anti-idiotype antibodies immunospecific for antibodies of the invention.

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According to another aspect of the invention, methods are provided for identifying novel anti-bacterial agents that modulate the function of gram negative bacteria virulence genes or gene products. Methods of the invention include screening potential agents for the ability to interfere with expression of virulence gene products encoded by the DNA sequences set forth in any one of SEQ ID NOS: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174, or species homologs thereof, or screening potential agents for the ability to interfere with biological function of a bacterial gene product encoded in whole or in part by a DNA sequence set forth in any one of SEQ ID NOS: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174, species homologs thereof, or the complementary strand thereof. followed by identifying agents that provide positive results in such screening assays. In particular, agents that interfere with the expression of virulence gene products include anti-sense polynucleotides and ribozymes that are complementary to the virulence gene sequences. The invention further embraces methods to modulate transcription of gene products of the invention through use of oligonucleotide-directed triplet helix formation.

Agents that interfere with the function of virulence gene products include variants of virulence gene products, binding partners of the virulence gene products and variants of such binding partners, and enzyme inhibitors (where the product is an enzyme).

Novel anti-bacterial agents identified by the methods described herein are provided, as well as methods for treating a subject suffering from infection with gram negative bacteria involving administration of such novel anti-bacterial agents in an amount effective to reduce bacterial presence.

Numerous additional aspects and advantages of the invention will become apparent to those skilled in the art upon consideration of the following detailed description of the invention which describes presently prepared embodiments thereof.

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DETAILED DESCRIPTION OF THE INVENTION

"Virulence genes," as used herein, are genes whose function or products are required for successful establishment and/or maintenance of bacterial infection in a host animal. Thus, virulence genes and/or the proteins encoded thereby are involved in pathogenesis in the host organism, but may not be necessary for growth.

"Signature-tagged mutagenesis (STM)," as used herein, is a method generally described in International Patent Publication No. WO 96/17951, incorporated herein by reference, and includes, for example, a method for identifying bacterial genes required for virulence in a murine model of bacteremia. In this method, bacterial strains that each have a random mutation in the genome are produced using transposon integration; each insertional mutation carries a different DNA signature tag which allows mutants to be differentiated from each other. The tags comprise 40 bp variable central regions flanked by invariant "arms" of 20 bp which allow the central portions to be co-amplified by polymerase chain reaction (PCR). Tagged mutant strains are assembled in microtiter dishes, then combined to form the "inoculum pool" for infection studies. At an appropriate time after inoculation, bacteria are isolated from the animal and pooled to form the "recovered pool." The tags in the recovered pool and the tags in the inoculum pool are separately amplified, labeled, and then used to probe filters arrayed with all of the different tags representing the mutants in the inoculum. Mutant strains with attenuated virulence are those which cannot be recovered from the infected animal, i.e., strains with tags that give hybridization signals when probed with tags from the inoculum pool but not when probed with tags from the recovered pool. In a variation of this method, nonradioactive detection methods such as chemiluminescence can be used

Signature-tagged mutagenesis allows a large number of insertional mutant strains to be screened simultaneously in a single animal for loss of virulence. Screening nineteen pools of mutant *P. multocida* strains resulted in the identification of more than 60 strains with reduced virulence, many of which were confirmed to be attenuated in virulence by subsequent determination of an approximate LD₅₀ for the individual mutants. Screening of *A. pleuropneumoniae* mutants resulted in identification of more than 100 strains having mutations in 35 different genes. Of these, mutations in 22 genes results in significantly attenuated *A. pleuropneumoniae* strains. The nucleotide sequence of the open reading frame disrupted by the transposon insertion was determined by sequencing both strands and an encoded amino acid sequence was deduced. Novelty of both the polynucleotide and amino acid sequences was determined by comparison of the sequences with DNA and protein database sequences. Knowledge of the virulence genes in these species permitted identification of species homologs in *P. (Mannheimia) haemolytica*.

The identification of bacterial, and more particularly *P. multocida A. pleuropneumoniae* and *P. (Mannheimia) haemolytica* virulence genes provides for microorganisms exhibiting reduced virulence (*i.e.*, attenuated strains), which are useful in vaccines. Such microorganisms include *Pasteurellaceae* mutants containing at least one functional mutation inactivating a gene represented by any one of SEQ ID NOS: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174. The worker of ordinary skill in the art will realize that a "functional mutation" may occur in protein coding regions of a gene of the invention, as well as in regulatory regions that modulate transcription of the virulence gene RNA.

The worker of ordinary skill will also appreciate that attenuated *P. multocida*, *A. pleuropneumoniae* and *P. (Mannheimia) haemolytica* strains of the invention include those bearing more than one functional mutation. More than one mutation may result in additive or synergistic degrees of attenuation. Multiple

mutations can be prepared by design or may fortuitously arise from a deletion event originally intended to introduce a single mutation. An example of an attenuated strain with multiple deletions is a *Salmonella typhimurium* strain wherein the *cya* and *crp* genes are functionally deleted. This mutant *S. typhimurium* strain has shown promise as a live vaccine.

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Identification of virulence genes in P. multocida, A. pleuropneumoniae and P. (Mannheimia) haemolytica can provide information regarding similar genes in other pathogenic species. As an example, identification of the aroA gene led to identification of conserved genes in a diverse number of pathogens, including Aeromonas hydrophila, Aeromonas salmonicida, Salmonella typhimurium, Salmonella enteritidis, Salmonella dublin, Salmonella gallanerum, Bordella pertussis, Yersinia entericolitica, Neisseria gonorrhoeae, and Bacillus anthracis. In many of these species, attenuated bacterial strains bearing mutations in the aroA gene have proven to be effective in vaccine formulations. Using the virulence genes sequences identified in P. multocida, similar or homologous genes can be identified in other organisms, particularly within the *Pasteurella* family, as well as A. pleuropneumoniae, P. (Mannheimia) haemolytica. and Haemophilus somnus. Likewise, identification of A. pleuropneumoniae virulence genes can permit identification of related genes in other organisms. Southern hybridization using the P. multocida, A. pleuropneumoniae and P. (Mannheimia) haemolytica genes as probes can identify these related genes in chromosomal libraries derived from other organisms. Alternatively, PCR can be equally effective in gene identification across species boundaries. As still another alternative, complementation of, for example, a P. multocida mutant with a chromosomal library from other species can also be used to identify genes having the same or related virulence activity. Identification of related virulence genes can therefore lead to production of an attenuated strain of the other organism which can be useful as still another vaccine formulation. Examples of P. multocida genes that have been demonstrated to exist in other species (e.g. P. (Mannheimia) haemolytica. A. pleuropneumoniae and H. somnus) include genes exbB, atpG, pnp, guaB and yigF.

Attenuated *P. multocida* strains identified using STM are insertional mutants wherein a virulence gene has been rendered non-functional through insertion of transposon sequences in either the open reading frame or regulatory DNA sequences. These insertional mutants still contain all of the genetic information required for bacterial virulence and can possibly revert to a pathogenic state by deletion of the inserted transposon. Therefore, in preparing a vaccine formulation, it is desirable to take the information gleaned from the attenuated strain and create a deletion mutant strain wherein some, most, or all of the virulence gene sequence is removed, thereby precluding the possibility that the bacteria will revert to a virulent state.

The vaccine properties of an attenuated insertional mutant identified using STM are expected to be the same or similar to those of a bacteria bearing a deletion in the same gene. However, it is possible that an insertion mutation may exert "polar" effects on adjoining gene sequences, and as a result, the insertion mutant may possess characteristic distinct from a mutant strain with a deletion in the same gene sequence. Deletion mutants can be constructed using any of a number of techniques well known and routinely practiced in the art.

In one example, a strategy using counterselectable markers can be employed which has commonly been utilized to delete genes in many bacteria. For a review, see, for example, Reyrat, et al., Infection and Immunity 66:4011-4017 (1998), incorporated herein by reference. In this technique, a double selection strategy is often employed wherein a plasmid is constructed encoding both a selectable and counterselectable marker, with flanking DNA sequences derived from both sides of the desired deletion. The selectable marker is used to select for bacteria in which the plasmid has integrated into the genome in the appropriate location and manner. The counterselecteable marker is used to select for the very small percentage of bacteria that have spontaneously eliminated the integrated plasmid. A fraction of these bacteria will then contain only the desired deletion with no other foreign DNA present. The key to the use of this technique is the availability of a suitable counterselectable marker.

In another technique, the *cre-lox* system is used for site specific recombination of DNA. The system consists of 34 base pair *lox* sequences that are recognized by the bacterial *cre* recombinase gene. If the *lox* sites are present in the DNA in an appropriate orientation, DNA flanked by the *lox* sites will be excised by the *cre* recombinase, resulting in the deletion of all sequences except for one remaining copy of the *lox* sequence. Using standard recombination techniques, it is possible to delete the targeted gene of interest in the *P. multocida*, *A. pleuropneumoniae* or *P. (Mannheimia) haemolytica* genome and to replace it with a selectable marker (e.g., a gene coding for kanamycin resistance) that is flanked by the *lox* sites. Transient expression (by electroporation of a suicide plasmid containing the *cre* gene under control of a promoter that functions in *P. multocida*, *A. pleuropneumoniae*, or *P. (Mannheimia) haemolytica*) of the *cre* recombinase should result in efficient elimination of the *lox* flanked marker. This process would result in a mutant containing the desired deletion mutation and one copy of the *lox* sequences.

In another approach, it is possible to directly replace a desired deleted sequence in the *P. multocida*, *A. pleuropneumoniae* or *P. (Mannheimia) haemolytica* genome with a marker gene, such as green fluorescent protein (GFP), β-galactosidase, or luciferase. In this technique, DNA segments flanking a desired deletion are prepared by PCR and cloned into a suicide (non-replicating) vector for *P. multocida*, *A. pleuropneumoniae*, or *P. (Mannheimia) haemolytica*. An expression cassette, containing a promoter active in *P. multocida*, *A. pleuropneumoniae*, or *P. (Mannheimia) haemolytica* and the appropriate marker gene, is cloned between the flanking sequences. The plasmid is introduced into wild-type *P. multocida*, *A. pleuropneumoniae* or *P. (Mannheimia) haemolytica*. Bacteria that incorporate and express the marker gene (probably at a very low frequency) are isolated and examined for the appropriate recombination event (*i.e.*, replacement of the wild type gene with the marker gene).

The reduced virulence of these organisms and their immunogenicity may be confirmed by administration to a subject animal. While it is possible for an avirulent microorganism of the invention to be administered alone, one or more of

such mutant microorganisms are preferably administered in a vaccine composition containing suitable adjuvant(s) and pharmaceutically acceptable diluent(s) or carrier(s). The carrier(s) must be "acceptable" in the sense of being compatible with the avirulent microorganism of the invention and not deleterious to the subject to be immunized. Typically, the carriers will be water or saline which will be sterile and pyrogen free. The subject to be immunized is a subject needing protection from a disease caused by a virulent form of *P. multocida*, *A. pleuropneumoniae*, *P. (Mannheimia) haemolytica* or other pathogenic microorganisms.

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It will be appreciated that the vaccine of the invention may be useful in the fields of human medicine and veterinary medicine. Thus, the subject to be immunized may be a human or other animal, for example, farm animals including cows, sheep, pigs, horses, goats and poultry (e.g., chickens, turkeys, ducks and geese) companion animals such as dogs and cats; exotic and/or zoo animals; and laboratory animals including mice, rats, rabbits, guinea pigs, and hamsters.

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The invention also provides polypeptides and corresponding polynucleotides required for P. multocida, A. pleuropneumoniae or P. (Mannheimia) haemolytica virulence. The invention includes both naturally occurring and non-naturally occurring polynucleotides and polypeptide products thereof. Naturally occurring virulence products include distinct gene and polypeptide species as well as corresponding species homologs expressed in organisms other than P. multocida, A. pleuropneumoniae, or P. (Mannheimia) haemolytica strains. Non-naturally occurring virulence products include variants of the naturally occurring products such as analogs and virulence products which include covalent modifications. In a preferred embodiment, the invention provides virulence polynucleotides comprising the sequences set forth in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174 and species homologs thereof, and polypeptides having amino acids sequences encoded by the polynucleotides.

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The present invention provides novel purified and isolated P. multocida, A. pleuropneumoniae and P. (Mannheimia) haemolytica polynucleotides (e.g., DNA sequences and RNA transcripts, both sense and complementary antisense strands) encoding the bacterial virulence gene products. DNA sequences of the invention include genomic and cDNA sequences as well as wholly or partially chemically synthesized DNA sequences. Genomic DNA of the invention comprises the protein coding region for a polypeptide of the invention and includes variants that may be found in other bacterial strains of the same species. "Synthesized," as used herein and is understood in the art, refers to purely chemical, as opposed to enzymatic, methods for producing polynucleotides. "Wholly" synthesized DNA sequences are therefore produced entirely by chemical means, and "partially" synthesized DNAs embrace those wherein only portions of the resulting DNA were produced by chemical means. Preferred DNA sequences encoding P. multocida virulence gene products are set out in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, and species homologs thereof. Preferred A. pleuropneumoniae DNA sequences encoding virulence gene products are set out in SEQ ID NOs: 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, and species homologs thereof. Preferred P. (Mannheimia) haemolytica virulence gene products are set out in SEQ ID NOs: 166, 168, 170, 172 and 174, and species homologs thereof. The worker of skill in the art will readily appreciate that the preferred DNA of the invention comprises a double stranded molecule, for example, molecules having the sequences set forth in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174 and species homologs thereof, along with the complementary molecule (the "noncoding strand" or "complement") having a sequence deducible from the sequence of SEQ ID NO: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53,

55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174, according to Watson-Crick base pairing rules for DNA. Also preferred are polynucleotides encoding the gene products encoded by any one of the polynucleotides set out in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174 and species homologs thereof. The invention further embraces species, preferably bacterial, homologs of the *P. multocida*, *A. pleuropneumoniae* and *P. (Mannheimia) haemolytica* DNA.

The polynucleotide sequence information provided by the invention makes possible the identification and isolation of polynucleotides encoding related bacterial virulence molecules by well known techniques including Southern and/or Northern hybridization, and polymerase chain reaction (PCR). Examples of related polynucleotides include polynucleotides encoding polypeptides homologous to a virulence gene product encoded by any one of the polynucleotides set out in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174, and species homologs thereof, and structurally related polypeptides sharing one or more biological and/or physical properties of a virulence gene product of the invention.

The invention also embraces DNA sequences encoding bacterial gene products which hybridize under moderately to highly stringent conditions to the non-coding strand, or complement, of any one of the polynucleotides set out in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146,

148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172 and 174, and species homologs thereof. DNA sequences encoding virulence polypeptides which would hybridize thereto but for the degeneracy of the genetic code are contemplated by the invention. Exemplary high stringency conditions include a final wash in buffer comprising 0.2X SSC/0.1% SDS, at 65°C to 75°C, while exemplary moderate stringency conditions include a final wash in buffer comprising 2X SSC/0.1% SDS, at 35°C to 45°C. It is understood in the art that conditions of equivalent stringency can be achieved through variation of temperature and buffer, or salt concentration as described in Ausubel, et al. (Eds.), Protocols in Molecular Biology, John Wiley & Sons (1994), pp. 6.0.3 to 6.4.10. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and the percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook, et al., (Eds.), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989), pp. 9.47 to 9.51.

Autonomously replicating recombinant expression constructions such as plasmid and viral DNA vectors incorporating virulence gene sequences are also provided. Expression constructs wherein virulence polypeptide-encoding polynucleotides are operatively linked to an endogenous or exogenous expression control DNA sequence and a transcription terminator are also provided. The virulence genes may be cloned by PCR, using *P. multocida* genomic DNA as the template. For ease of inserting the gene into expression vectors, PCR primers are chosen so that the PCR-amplified gene has a restriction enzyme site at the 5' end preceding the initiation codon ATG, and a restriction enzyme site at the 3' end after the termination codon TAG, TGA or TAA. If desirable, the codons in the gene are changed, without changing the amino acids, according to *E. coli* codon preference described by Grosjean and Fiers, *Gene, 18*:199-209 (1982), and Konigsberg and Godson, *Proc. Natl. Acad. Sci. (USA), 80*:687-691 (1983). Optimization of codon usage may lead to an increase in the expression of the gene product when produced in *E. coli*. If the gene product is to be produced extracellularly, either in the periplasm of

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E. coli or other bacteria, or into the cell culture medium, the gene is cloned without its initiation codon and placed into an expression vector behind a signal sequence.

According to another aspect of the invention, host cells are provided, including procaryotic and eukaryotic cells, either stably or transiently transformed, transfected, or electroporated with polynucleotide sequences of the invention in a manner which permits expression of virulence polypeptides of the invention. Expression systems of the invention include bacterial, yeast, fungal, viral, invertebrate, and mammalian cells systems. Host cells of the invention are a valuable source of immunogen for development of antibodies specifically immunoreactive with the virulence gene product. Host cells of the invention are conspicuously useful in methods for large scale production of virulence polypeptides wherein the cells are grown in a suitable culture medium and the desired polypeptide products are isolated from the cells or from the medium in which the cells are grown by, for example, immunoaffinity purification or any of the multitude of purification techniques well known and routinely practiced in the art. Any suitable host cell may be used for expression of the gene product, such as E. coli, other bacteria, including P. multocida, Bacillus and S. aureus, yeast, including Pichia pastoris and Saccharomyces cerevisiae, insect cells, or mammalian cells, including CHO cells, utilizing suitable vectors known in the art. Proteins may be produced directly or fused to a peptide or polypeptide, and either intracellularly or extracellularly by secretion into the periplasmic space of a bacterial cell or into the cell culture medium. Secretion of a protein requires a signal peptide (also known as pre-sequence); a number of signal sequences from prokaryotes and eukaryotes are known to function for the secretion of recombinant proteins. During the protein secretion process, the signal peptide is removed by signal peptidase to yield the mature protein.

To simplify the protein purification process, a purification tag may be added either at the 5° or 3° end of the gene coding sequence. Commonly used purification tags include a stretch of six histidine residues (U.S. Patent Nos. 5,284,933 and 5,310,663), a streptavidin-affinity tag described by Schmidt and Skerra, *Protein Engineering*, 6:109-122 (1993), a FLAG peptide [Hopp *et al.*, *Biotechnology*, 6:1205-

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1210 (1988)], glutathione S-transferase [Smith and Johnson, *Gene*, 67:31-40 (1988)], and thioredoxin [LaVallie et al., *Bio/Technology*, 11:187-193 (1993)]. To remove these peptide or polypeptides, a proteolytic cleavage recognition site may be inserted at the fusion junction. Commonly used proteases are factor Xa, thrombin, and enterokinase.

The invention also provides purified and isolated P. multocida, A. pleuropneumoniae and P. (Mannheimia) haemolytica virulence polypeptides encoded by a polynucleotide of the invention. Presently preferred are polypeptides comprising the amino acid sequences encoded by any one of the polynucleotides set out in SEO ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 164, 166, 168, 170, 172 and 174, and species homologs thereof. The invention embraces virulence polypeptides encoded by a DNA selected from the group consisting of: a) the DNA sequence set out in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 164, 166, 168, 170, 172, and 174 and species homologs thereof; b) DNA molecules encoding P. multocida, A. pleuropneumoniae or P. (Mannheimia) haemolytica. polypeptides encoded by any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 164, 166, 168, 170, 172, and 174, and species homologs thereof; and c) a DNA molecule, encoding a virulence gene product, that hybridizes under moderately stringent conditions to the DNA of (a) or (b).

The invention also embraces polypeptides that have at least about 99%, at least about 95%, at least about 90%, at least about 85%, at least about 80%, at least about 75%, at least about 70%, at least about 65%, at least about 60%, at least

about 55%, and at least about 50% identity and/or homology to the preferred polypeptides of the invention. Percent amino acid sequence "identity" with respect to the preferred polypeptides of the invention is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the virulence gene product sequence after aligning both sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Percent sequence "homology" with respect to the preferred polypeptides of the invention is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in one of the virulence polypeptide sequences after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and also considering any conservative substitutions as part of the sequence identity. Conservative substitutions can be defined as set out in Tables A and B.

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Table A
Conservative Substitutions I

	SIDE CHAIN	CHARACTERISTIC	AMINO ACID
20	Aliphatic	Non-polar	GAP
			ILV
		Polar - uncharged	CSTM
			NQ
		Polar - charged	DE
25		·	ΚR
	Aromatic		HFWY
	Other		NODE

Polypeptides of the invention may be isolated from natural bacterial cell sources or may be chemically synthesized, but are preferably produced by recombinant procedures involving host cells of the invention. Virulence gene products of the invention may be full length polypeptides, biologically active fragments, or variants thereof which retain specific biological or immunological activity. Variants may comprise virulence polypeptide analogs wherein one or more

of the specified (*i.e.*, naturally encoded) amino acids is deleted or replaced or wherein one or more non-specified amino acids are added: (1) without loss of one or more of the biological activities or immunological characteristics specific for the virulence gene product; or (2) with specific disablement of a particular biological activity of the virulence gene product. Deletion variants contemplated also include fragments lacking portions of the polypeptide not essential for biological activity, and insertion variants include fusion polypeptides in which the wild-type polypeptide or fragment thereof have been fused to another polypeptide.

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Variant virulence polypeptides include those wherein conservative substitutions have been introduced by modification of polynucleotides encoding polypeptides of the invention. Conservative substitutions are recognized in the art to classify amino acids according to their related physical properties and can be defined as set out in Table A (from WO 97/09433, page 10, published March 13, 1997 (PCT/GB96/02197, filed 9/6/96). Alternatively, conservative amino acids can be grouped as defined in Lehninger, [Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp.71-77] as set out in Table B.

Table B
Conservative Substitutions II

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20	SIDE CHAIN CHARACTERISTIC	AMINO ACID	
	Non-polar (hydrophobic)		
25	A. Aliphatic:	ALIVP	
	B. Aromatic:	F W	
	C. Sulfur-containing:	M	
	D. Borderline:	G	
	Uncharged-polar		
30	A. Hydroxyl:	STY	
	B. Amides:	ΝQ	
	C. Sulfhydryl:	C	
	D. Borderline:	G	
	Positively Charged (Basic):	KRH	
35	Negatively Charged (Acidic):	DE	

Variant virulence products of the invention include mature virulence gene products, *i.e.*, wherein leader or signal sequences are removed, having additional amino terminal residues. Virulence gene products having an additional methionine residue at position -1 are contemplated, as are virulence products having additional methionine and lysine residues at positions -2 and -1. Variants of these types are particularly useful for recombinant protein production in bacterial cell types. Variants of the invention also include gene products wherein amino terminal sequences derived from other proteins have been introduced, as well as variants comprising amino terminal sequences that are not found in naturally occurring proteins.

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The invention also embraces variant polypeptides having additional amino acid residues which result from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide as a fusion protein with glutathione-S-transferase (GST) provide the desired polypeptide having an additional glycine residue at position -1 following cleavage of the GST component from the desired polypeptide. Variants which result from expression using other vector systems are also contemplated.

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Also comprehended by the present invention are antibodies (e.g., monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, humanized, human, and CDR-grafted antibodies, including compounds which include CDR sequences which specifically recognize a polypeptide of the invention) and other binding proteins specific for virulence gene products or fragments thereof. The term "specific for" indicates that the variable regions of the antibodies of the invention recognize and bind a virulence polypeptide exclusively (i.e., are able to distinguish a single virulence polypeptides from related virulence polypeptides despite sequence identity, homology, or similarity found in the family of polypeptides), but may also interact with other proteins (for example, S. aureus protein A or other antibodies in ELISA techniques) through interactions with sequences outside the variable region of the antibodies, and in particular, in the constant region of the molecule. Screening assays to determine binding specificity of an antibody of the invention are well known and routinely practiced in the art. For a comprehensive discussion of such assays, see

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Harlow et al. (Eds), Antibodies A Laboratory Manual; Cold Spring Harbor Laboratory; Cold Spring Harbor, NY (1988), Chapter 6. Antibodies that recognize and bind fragments of the virulence polypeptides of the invention are also contemplated, provided that the antibodies are first and foremost specific for, as defined above, a virulence polypeptide of the invention from which the fragment was derived.

The DNA and amino acid sequence information provided by the present invention also makes possible the systematic analysis of the structure and function of the virulence genes and their encoded gene products. Knowledge of a polynucleotide encoding a virulence gene product of the invention also makes available anti-sense polynucleotides which recognize and hybridize to polynucleotides encoding a virulence polypeptide of the invention. Full length and fragment anti-sense polynucleotides are provided. The worker of ordinary skill will appreciate that fragment anti-sense molecules of the invention include (i) those which specifically recognize and hybridize to a specific RNA (as determined by sequence comparison of DNA encoding a virulence polypeptide of the invention to DNA encoding other known molecules) as well as (ii) those which recognize and hybridize to RNA encoding variants of the family of virulence proteins. Antisense polynucleotides that hybridize to RNA encoding other members of the virulence family of proteins are also identifiable through sequence comparison to identify characteristic, or signature, sequences for the family of molecules.

The invention further contemplates methods to modulate gene expression through use of ribozymes. For a review, see Gibson and Shillitoe, *Mol. Biotech.* 7:125-137 (1997). Ribozyme technology can be utilized to inhibit translation of mRNA in a sequence specific manner through (i) the hybridization of a complementary RNA to a target mRNA and (ii) cleavage of the hybridized mRNA through nuclease activity inherent to the complementary strand. Ribozymes can be identified by empirical methods but more preferably are specifically designed based on accessible sites on the target mRNA [Bramlage, *et al.*, *Trends in Biotech 16*:434-438 (1998)]. Delivery of ribozymes to target cells can be accomplished using either

exogenous or endogenous delivery techniques well known and routinely practiced in the art. Exogenous delivery methods can include use of targeting liposomes or direct local injection. Endogenous methods include use of viral vectors and non-viral plasmids.

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Ribozymes can specifically modulate expression of virulence genes when designed to be complementary to regions unique to a polynucleotide encoding a virulence gene product. "Specifically modulate" therefore is intended to mean that ribozymes of the invention recognizes only a single polynucleotide. Similarly, ribozymes can be designed to modulate expression of all or some of a family of proteins. Ribozymes of this type are designed to recognize polynucleotide sequences conserved in all or some of the polynucleotides which encode the family of proteins.

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The invention further embraces methods to modulate transcription of a virulence gene of the invention through use of oligonucleotide-directed triplet helix formation. For a review, see Lavrovsky, et al., Biochem. Mol. Med. 62:11-22 (1997). Triplet helix formation is accomplished using sequence specific oligonucleotides which hybridize to double stranded DNA in the major groove as defined in the Watson-Crick model. Hybridization of a sequence specific oligonucleotide can thereafter modulate activity of DNA-binding proteins, including, for example, transcription factors and polymerases. Preferred target sequences for hybridization include transcriptional regulatory regions that modulate virulence gene product expression. Oligonucleotides which are capable of triplet helix formation are also useful for site-specific covalent modification of target DNA sequences. Oligonucleotides useful for covalent modification are coupled to various DNA damaging agents as described in Lavrovsky, et al. [supra].

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The identification of *P. multocida*, *A. pleuropneumoniae* and *P. (Mannheimia) haemolytica* virulence genes renders the genes and gene products useful in methods for identifying anti-bacterial agents. Such methods include assaying potential agents for the ability to interfere with expression of virulence gene products represented by the DNA sequences set forth in any one of SEQ ID NOS: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68,

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70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174 and species homologs thereof (i.e., the genes represented by DNA sequences of SEQ ID NOS: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174 encode the virulence gene product, or the DNA sequences of SEQ ID NOS: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174 are adjacent the gene encoding the virulence gene product, or are involved in regulation of expression of the virulence gene product), or assaying potential agents for the ability to interfere with the function of a bacterial gene product encoded in whole or in part by a DNA sequence set forth in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174, species homologs thereof, or the complementary strand thereof, followed by identifying agents that are positive in such assays. Polynucleotides and polypeptides useful in these assays include not only the genes and encoded polypeptides as disclosed herein, but also variants thereof that have substantially the same activity as the wild-type genes and polypeptides.

The virulence gene products produced by the methods described above are used in high throughput assays to screen for inhibitory agents. The sources for potential agents to be screened are chemical compound libraries, fermentation media of *Streptomycetes*, other bacteria and fungi, and cell extracts of plants and other vegetations. For proteins with known enzymatic activity, assays are established based

on the activity, and a large number of potential agents are screened for ability to inhibit the activity. For proteins that interact with another protein or nucleic acid, binding assays are established to measure such interaction directly, and the potential agents are screened for ability to inhibit the binding interaction.

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The use of different assays known in the art is contemplated according to this aspect of the invention. When the function of the virulence gene product is known or predicted by sequence similarity to a known gene product, potential inhibitors can be screened in enzymatic or other types of biological and/or bjochemical assays keyed to the function and/or properties of the gene product. When the virulence gene product is known or predicted by sequence similarity to a known gene product to interact with another protein or nucleic acid, inhibitors of the interaction can be screened directly in binding assays. The invention contemplates a multitude of assays to screen and identify inhibitors of binding by the virulence gene product. In one example, the virulence gene product is immobilized and interaction with a binding partner is assessed in the presence and absence of a putative inhibitor compound. In another example, interaction between the virulence gene product and its binding partner is assessed in a solution assay, both in the presence and absence of a putative inhibitor compound. In both assays, an inhibitor is identified as a compound that decreases binding between the virulence gene product and its binding partner. Other assays are also contemplated in those instances wherein the virulence gene product binding partner is a protein. For example, variations of the di-hybrid assay are contemplated wherein an inhibitor of protein/protein interactions is identified by detection of a positive signal in a transformed or transfected host cell as described in PCT publication number WO 95/20652, published August 3, 1995.

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Candidate inhibitors contemplated by the invention include compounds selected from libraries of potential inhibitors. There are a number of different libraries used for the identification of small molecule modulators, including: (1) chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of random peptides, oligonucleotides or organic molecules. Chemical libraries consist of structural analogs of known compounds or compounds that are

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identified as "hits" or "leads" via natural product screening. Natural product libraries are collections of microorganisms, animals, plants, or marine organisms which are used to create mixtures for screening by: (1) fermentation and extraction of broths from soil, plant or marine microorganisms or (2) extraction of plants or marine organisms. Natural product libraries include polyketides, non-ribosomal peptides. and variants (non-naturally occurring) thereof. For a review, see Science 282:63-68 (1998). Combinatorial libraries are composed of large numbers of peptides, oligonucleotides, or organic compounds as a mixture. They are relatively easy to prepare by traditional automated synthesis methods, PCR, cloning, or proprietary synthetic methods. Of particular interest are peptide and oligonucleotide combinatorial libraries. Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created therefrom, see Myers, Curr. Opin. Biotechnol. 8:701-707 (1997). Identification of modulators through use of the various libraries described herein permits modification of the candidate "hit" (or "lead") to optimize the capacity of the "hit" to modulate activity.

Still other candidate inhibitors contemplated by the invention can be designed and include soluble forms of binding partners, as well as binding partners as chimeric, or fusion, proteins. Binding partners as used herein broadly encompasses antibodies, antibody fragments, and modified compounds comprising antibody domains that are immunospecific for the expression product of the identified virulence gene.

Other assays may be used when a binding partner (i.e., ligand) for the virulence gene product is not known, including assays that identify binding partners of the target protein through measuring direct binding of test binding partner to the target protein, and assays that identify binding partners of target proteins through affinity ultrafiltration with ion spray mass spectroscopy/HPLC methods or other physical and analytical methods. Alternatively, such binding interactions are evaluated indirectly using the yeast two-hybrid system described in Fields and Song, *Nature*, 340:245-246 (1989), and Fields and Sternglanz, *Trends in Genetics*, 10:286-292 (1994), both of

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which are incorporated herein by reference. The two-hybrid system is a genetic assay for detecting interactions between two proteins or polypeptides. It can be used to identify proteins that bind to a known protein of interest, or to delineate domains or residues critical for an interaction. Variations on this methodology have been developed to clone genes that encode DNA-binding proteins, to identify peptides that bind to a protein, and to screen for drugs. The two-hybrid system exploits the ability of a pair of interacting proteins to bring a transcription activation domain into close proximity with a DNA-binding domain that binds to an upstream activation sequence (UAS) of a reporter gene, and is generally performed in yeast. The assay requires the construction of two hybrid genes encoding (1) a DNA-binding domain that is fused to a first protein and (2) an activation domain fused to a second protein. The DNAbinding domain targets the first hybrid protein to the UAS of the reporter gene; however, because most proteins lack an activation domain, this DNA-binding hybrid protein does not activate transcription of the reporter gene. The second hybrid protein, which contains the activation domain, cannot by itself activate expression of the reporter gene because it does not bind the UAS. However, when both hybrid proteins are present, the noncovalent interaction of the first and second proteins tethers the activation domain to the UAS, activating transcription of the reporter gene. When the virulence gene product (the first protein, for example) is already known to interact with another protein or nucleic acid, this assay can be used to detect agents that interfere with the binding interaction. Expression of the reporter gene is monitored as different test agents are added to the system; the presence of an inhibitory agent results in lack of a reporter signal.

When the function of the virulence gene product is unknown and no ligands are known to bind the gene product, the yeast two-hybrid assay can also be used to identify proteins that bind to the gene product. In an assay to identify proteins that bind to the first protein (the target protein), a large number of hybrid genes each encoding different second proteins are produced and screened in the assay. Typically, the second protein is encoded by a pool of plasmids in which total cDNA or genomic DNA is ligated to the activation domain. This system is applicable to a wide variety

of proteins, and it is not even necessary to know the identity or function of the second binding protein. The system is highly sensitive and can detect interactions not revealed by other methods; even transient interactions may trigger transcription to produce a stable mRNA that can be repeatedly translated to yield the reporter protein.

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Other assays may be used to search for agents that bind to the target protein. One such screening method to identify direct binding of test ligands to a target protein is described in U.S. Patent No. 5,585,277, incorporated herein by reference. This method relies on the principle that proteins generally exist as a mixture of folded and unfolded states, and continually alternate between the two states. When a test ligand binds to the folded form of a target protein (i.e., when the test ligand is a ligand of the target protein), the target protein molecule bound by the ligand remains in its folded state. Thus, the folded target protein is present to a greater extent in the presence of a test ligand which binds the target protein, than in the absence of a ligand. Binding of the ligand to the target protein can be determined by any method which distinguishes between the folded and unfolded states of the target protein. The function of the target protein need not be known in order for this assay to be performed. Virtually any agent can be assessed by this method as a test ligand, including, but not limited to, metals, polypeptides, proteins, lipids, polysaccharides, polynucleotides and small organic molecules.

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Another method for identifying ligands for a target protein is described in Wieboldt et al., Anal. Chem., 69:1683-1691 (1997), incorporated herein by reference. This technique screens combinatorial libraries of 20-30 agents at a time in solution phase for binding to the target protein. Agents that bind to the target protein are separated from other library components by centrifugal ultrafiltration. The specifically selected molecules that are retained on the filter are subsequently liberated from the target protein and analyzed by HPLC and pneumatically assisted electrospray (ion spray) ionization mass spectroscopy. This procedure selects library components with the greatest affinity for the target protein, and is particularly useful for small molecule libraries.

The inhibitors/binders identified by the initial screens are evaluated for their effect on virulence in *in vivo* mouse models of *P. multocida* infections. Models of bacteremia, endocarditis, septic arthritis, soft tissue abscess, or pneumonia may be utilized. Models involving use of other animals are also comprehended by the invention. For example, rabbits can be challenged with a wild type *P. multocida* strain before or after administration of varying amounts of a putative inhibitor/binder compound. Control animals, administered only saline instead of putative inhibitor/binder compound provide a standard by which deterioration of the test animal can be determined. Other animal models include those described in the Animal and Plant Health Inspection Sevice, USDA, January 1, 1994 Edition, §§113.69-113.70; Panciera and Corstvet, *Am. J. Vet. Res.* 45:2532-2537; Ames, *et al., Can. J. Comp. Med.* 49:395-400 (1984); and Mukkur, *Infection and Immunity 18*:583-585 (1977). Inhibitors/binders that interfere with bacterial virulence are can prevent the establishment of an infection or reverse the outcome of an infection once it is established.

Any adjuvant known in the art may be used in the vaccine composition, including oil-based adjuvants such as Freund's Complete Adjuvant and Freund's Incomplete Adjuvant, mycolate-based adjuvants (e.g., trehalose dimycolate), bacterial lipopolysaccharide (LPS), peptidoglycans (i.e., mureins, mucopeptides, or glycoproteins such as N-Opaca, muramyl dipeptide [MDP], or MDP analogs), proteoglycans (e.g., extracted from *Klebsiella pneumoniae*), streptococcal preparations (e.g., OK432), BiostimTM (e.g., 01K2), the "Iscoms" of EP 109 942, EP 180 564 and EP 231 039, aluminum hydroxide, saponin, DEAE-dextran, neutral oils (such as miglyol), vegetable oils (such as arachis oil), liposomes, Pluronic® polyols, the Ribi adjuvant system (see, for example GB-A-2 189 141), or interleukins, particularly those that stimulate cell mediated immunity. An alternative adjuvant consisting of extracts of *Amycolata*, a bacterial genus in the order Actinomycetales, has been described in U.S. Patent No. 4,877,612. Additionally, proprietary adjuvant mixtures are commercially available. The adjuvant used will depend, in part, on the

recipient organism. The amount of adjuvant to administer will depend on the type and size of animal. Optimal dosages may be readily determined by routine methods.

The vaccine compositions optionally may include vaccine-compatible pharmaceutically acceptable (i.e., sterile and non-toxic) liquid, semisolid, or solid diluents that serve as pharmaceutical vehicles, excipients, or media. Any diluent known in the art may be used. Exemplary diluents include, but are not limited to, polyoxyethylene sorbitan monolaurate, magnesium stearate, methyl- and propylhydroxybenzoate, talc, alginates, starches, lactose, sucrose, dextrose, sorbitol, mannitol, gum acacia, calcium phosphate, mineral oil, cocoa butter, and oil of theobroma.

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The vaccine compositions can be packaged in forms convenient for delivery. The compositions can be enclosed within a capsule, caplet, sachet, cachet, gelatin, paper, or other container. These delivery forms are preferred when compatible with entry of the immunogenic composition into the recipient organism and, particularly, when the immunogenic composition is being delivered in unit dose form. The dosage units can be packaged, e.g., in tablets, capsules, suppositories or cachets.

The vaccine compositions may be introduced into the subject to be immunized by any conventional method including, e.g., by intravenous, intradermal, intramuscular, intramammary, intraperitoneal, or subcutaneous injection; by oral, sublingual, nasal, anal, or vaginal, delivery. The treatment may consist of a single dose or a plurality of doses over a period of time.

The invention also comprehends use of an attenuated bacterial strain of the invention for manufacture of a vaccine medicament to prevent or alleviate bacterial infection and/or symptoms associated therewith. The invention also provides use of inhibitors of the invention for manufacture of a medicament to prevent or alleviate bacterial infection and/or symptoms associated therewith.

The present invention is illustrated by the following examples. Example 1 describes constructions of *P. multocida* mutants. Example 2 relates to screening for *P. multocida* mutants. Example 3 addresses methods to determine

virulence of the *P. multocida* mutants. Example 4 describes cloning of *P. multocida* virulence genes. Example 5 addresses identification of genes in other species related to *P. multocida* virulence genes. Example 6 describes construction of *A. pleuropneumoniae* mutants. Example 7 addresses screening for attenuated *A. pleuropneumoniae* mutants. Example 8 relates to identification of *A. pleuropneumoniae* virulence genes. Example 9 describes competition challenge of *A. pleuropneumoniae* mutants and wild type bacteria. Example 10 characterizes *A. pleuropneumoniae* genes identified. Example 11 addresses efficacy of *A. pleuropneumoniae* mutant to protect against wild type bacterial challenge. Example 12 describes identification of species homolog virulence genes in *P. (Mannheimia) haemolytica*.

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Example 1 Construction of a Library of Tagged-Transposon *P. multocida* Mutants

A library of tagged-transposon mutants was constructed in parental vector pLOF/Km [Herrero, et al., J Bacteriol. 172:6557-67 (1990)] which has previously been demonstrated to be functional and random in P. multocida [Lee, et al., Vet Microbiol. 50:143-8 (1996)]. Plasmid pLOF/Km was constructed as a modification of suicide vector pGP704 and included a transposase gene under control of the Tac promoter as well as the mini-Tn10 transposable element encoding kanamycin resistance. Plasmid pTEF-1 was constructed as described below by modifying pLOF/Km to accept sequence tags which contained a semi-random [NK]₃₅ sequence.

Plasmid pLOF/Km was first modified to eliminate the unique *Kpn*I restriction site in the multiple cloning region and then to introduce a new *Kpn*I site in the mini-Tn10 region. The plasmid was digested with *Kpn*I and the resulting overhanging ends were filled in with Klenow polymerase according to manufacturer's suggested protocol. Restriction digests and ligations described herein were performed according to manufacturer's suggested protocols (Gibco BRL, Gaithersburg, MD and Boehringer Mannheim, Indianapolis, IN). The blunt end product was self-ligated to

produce a plasmid designated pLOF/Km--KpnI which was transformed into E.coli DH5α:λpir for amplification. E.coli DH5α: (λpir φ80dlacZΔM15, recA1, endA1, gyrA96, thi-1, hsdR17(r_k, m_k, supE44, relA1, deoR, Δ(lacZYA-argF)U169, was propagated at 37°C in Luria-Bertani (LB) medium. Plasmids were prepared using QIAGEN SpinPreps from QIAGEN Inc. (Santa Clarita, CA) and digested with SfiI which cuts at a unique site within the mini-Tn10 transposable element. A SfiI-KpnI-SfiI adaptor was prepared by annealing oligonucleotides TEF1 (SEQ ID NO: 86) and TEF3 (SEQ ID NO: 87) and the resulting double-stranded adapter was ligated into the SfiI site to create plasmid pTEF-1. Oligonucleotides TEF1 and TEF3 (as well as all other oligonucleotides described herein) were synthesized by Genosys Biotechnologies (The Woodlands, TX).

TEF1 5'-AGGCCGGTACCGGCCGCCT SEQ ID NO: 86

TEF3 5'-CGGCCGGTACCGGCCTAGG SEQ ID NO: 87

Unique sequence tags for insertion into the *Kpn*I site of pTEF-1 were prepared as follows. PCR was carried out to generate double stranded DNA tags using a GeneAmp XL PCR Kit (PE Applied Biosystems, Foster City, CA) under conditions including 250 µM each dNTP, 1.5 mM Mg(OAc)₂, 100 pmol each primer TEF14 (SEQ ID NO: 88) and TEF15 (SEQ ID NO: 89), 1 ng TEF26 (SEQ ID NO: 90) as template DNA and 2.5 units recombinant *Tth* DNA Polymerase XL.

TEF14 5'-CATGGTACCCATTCTAAC SEQ ID NO: 88

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TEF15 5'-CTAGGTACCTACAACCTC SEQ ID NO: 89

TEF26 SEQ ID NO: 90

5'-CTAGGTACCTACAACCTCAAGCTT-[NK]₃₅AAGCTTGGTTAGAATGGGTACCATG

Reaction conditions included an initial incubation at 95°C for one minute, followed by thirty cycles of 30 seconds at 95°C, 45 seconds at 45°C, and 15 seconds at 72°C, followed by a final incubation at 72°C for two minutes. The PCR products were digested with *Kpn*I and purified using a QIAGEN Nucleotide Removal Kit (QIAGEN, Inc., Chatsworth, GA) according to the manufacturer's suggested protocol. The unique tag sequences were ligated into the mini-Tn10 element of linearized pTEF-1, previously digested with *Kpn*I and dephosphorylated with calf intestinal alkaline phosphatase (Boehringer Mannheim) using standard procedures. The resulting plasmid library was transformed into *E.coli* DH5α:λpir. Colony blot analysis was performed according to the DIG User's Guide (Boehringer-Mannheim) with hybridization and detection performed as follows.

Hybridizations were essentially performed according to the Genius Non-Radioactive User's Guide (Boehringer Mannheim Biochemicals), the product sheet for the DIG-PCR labeling kit (Boehringer Mannheim Biochemicals), and the product sheet for CSPD (Boehringer Mannheim Biochemicals). For preparation of probes, a 100 μl primary PCR reaction was set up using Amplitaq PCR buffer (PE Applied Biosystems), 200 μM dNTPs, 140 pmol each of primers TEF5 (SEQ ID NO: 91) and TEF6 (SEQ ID NO: 92), 2 mM MgCl₂, 2.5 units Amplitaq (PE Applied Biosystems) and 1 ng of plasmid DNA.

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TEF5 5'-TACCTACAACCTCAAGCT SEQ ID NO: 91

TEF6 5'-TACCCATTCTAACCAAGC SEQ ID NO: 92

Cycle conditions included an initial incubation at 95°C for two minutes, followed by 35 cycles of 95°C for 30 seconds, 50°C for 45 seconds, 72°C for 15 seconds and a final incubation at 72°C for three minutes. The amplification products were separated using electrophoresis on a 2% - 3:1 NuSieve GTG (FMC BioProducts, Rockland, ME, USA):Agarose gel and the 109 bp product was excised and purified. Gel extractions were carried out using a QIAGEN Gel Extraction kit (QIAGEN).

Approximately 15 ng of the primary product was labeled in a 50 μ l PCR reaction using the DIG PCR Kit, 50 pmol each of primers TEF24 and TEF25, and a 1:1 mix of DIG Probe Synthesis Mix with 2 mM dNTP stock solution.

5 TEF24 5'-TACCTACAACCTCAAGCTT SEQ ID NO: 93

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TEF25 5'-TACCCATTCTAACCAAGCTT SEQ ID NO: 94

PCR conditions included an initial incubation at 95°C for four minutes, followed by 25 cycles of 95°C for 30 seconds, 50°C for 45 seconds, 72°C for 15 seconds and a final incubation at 72°C for three minutes. The labeled PCR product was digested with *Hin*dIII in a total reaction volume of 90 µl and purified from the constant primer arms using a 2% - 3:1 NuSieve GTG (FMC BioProducts):Agarose gel. The region containing the labeled variable tag was excised and the entire gel slice was dissolved and denatured in 10 ml of DIG EasyHyb at 95°C for ten minutes.

Dot blots were prepared using a Hybond P-N+ membrane (Amersham-Pharmacia Biotech). Target DNA for each tag was prepared in 96 well plates using approximately 30 ng of PCR product. An equal volume of 0.1 N NaOH was added to denature the sample and each sample was applied to the membrane with minimal vacuum using a Minifold ITM Dot-Blot Apparatus from Schleicher and Schuell (Keene, NH, USA). Each well was washed with 150 µl of Neutralization Solution (0.5 M Tris /3 M NaCl, pH 7.5) and 150 µl of 2X SSC. Membranes were UV-crosslinked in a Stratalinker (Stratagene, La Jolla, CA, USA) and prehybridized for one hour in 20 mls DIG EasyHyb Buffer at 42°C. The denatured probe was added and hybridization carried out overnight at 42°C. The membrane was washed two times in 2X SSC containing 0.1% SDS for five minutes each wash. Two high stringency washes were performed in 50 ml of pre-warmed 0.1X SSC buffer containing 0.1% SDS at 68°C for 15 minutes before proceeding with standard Genius Detection protocols (Genius Manual).

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It is desirable to use a non-radioactive detection system for safety, lower cost, ease of use, and reduction of hazardous materials. In initial experiments using similar procedures previously described [Mei, et al., Mol Microbiol. 26:399-407 (1997)], unacceptable background levels of hybridization were obtained in negative controls. In order to decrease background, tag length was increased by 30 bp to a total of 70, amplification primers were lengthened to include all sequence flanking the variable region, a lower concentration of dig-dUTP was used, and the conserved sequences flanking the sequence tag region were removed by gel purification. Most significantly, PCR was used to generate [NK]₃₅ sequence tags as the target DNA in dot blots rather than the entire plasmids containing the tagged transposons after detecting background hybridization from the transposon itself. Using these modifications background was eliminated making chemiluminescent/non-radioactive screening more effective.

Approximately four hundred different transformants resulting from the ligation of pTEF-1 with the PCR generated sequence tags were screened by colony blot and the 96 strongest hybridizing colonies were assembled into microtiter plates for further use. Even though the likelihood of duplicated tags was very low, half of the plate of master tags was probed against the other to confirm that no tags were duplicated. The plasmids containing these tags were purified and transformed into E.coli S17-1:λpir (pir, recA, thi, pro, hsd, (r-m+), RP4-2, (Tc::Mu), (Km::Tn7), [TmpR], [SmR]), and the transformed bacteria propagated at 37°C in Luria-Bertani (LB) medium. Each of the 96 E.coli S17-1: Apir transformants containing the tagged plasmid pTEF-1 was used in conjugative matings to generate transposon mutants of P. multocida. P. multocida strain TF5 is a spontaneous nalidixic acid resistant mutant derived from UC6731, a bovine clinical isolate. P. multocida strains were grown on brain heart infusion (BHI) media (Difco Laboratories, Detroit, MI, USA) at 37°C and in 5% CO₂ when grown on plates. Matings were set up by growing each E.coli S17-1:λpir /pTEF1:[NK]₃₅ clone and the TF5 strain to late log phase. Fifty μl of culture for each tagged-pTEF-1 clone was mixed with 200 µl of the TF5 culture and 50 µl of each mating mixture was spotted onto 0.22 TM filters previously placed on BHI plates

containing 100 mM IPTG and 10 mM MgSO₄. Following overnight incubation at 37°C with 5% CO₂, mating mixtures were washed off of each filter into 3 ml of PBS and 25 µl of each was plated onto BHIN⁵⁰K¹⁰⁰ plates. Following selective overnight growth, colonies were assembled into microtiter plates by toothpick transfer into 200 µl BHIN⁵⁰K⁵⁰ making sure that each well in a microtiter plate always contained a transposon mutant with the same sequence tag. Following overnight growth, 50 µl of 75% glycerol was added to each well and plates were stored frozen at -80°C.

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Nineteen pools were assembled by transferring the transposon mutants to microtiter plates making sure that each well contained a transposon mutant with the appropriate tag for that well. In other words, a specific well in each microtiter plate always contained a transposon mutant with the same sequence tag even though the location of the transposon within those mutants may be different.

Example 2 Murine Screening for Attenuated *P. multocida* Mutants

Nineteen pools of Pasteurella multocida transposon mutants were screened using a murine model of septicemia. Frozen plates of pooled P. multocida transposon mutants were removed from -80°C storage and subcultured by transferring 10 µl from each well to a new 96 well round bottom plate (Corning Costar, Cambridge, MA, USA) containing 200 µl of brain heart infusion (DIFCO) with 50 μg/ml nalidixic acid (Sigma) and 50 μg/ml kanamycin (Sigma) (BHIN⁵⁰K⁵⁰). Plates were incubated without shaking overnight at 37°C in 5% CO₂. Overnight plates were subcultured by transferring 10 µl from each well to a new flat bottomed 96-well plate (Corning Costar) containing 100 µl of BHI per well and incubating at 37°C with shaking at approximately 150 rpm. The OD_{540} was monitored using a micro-titer plate reader. At an OD₅₄₀ of approximately 0.2 to 0.25, each plate was pooled to form the "input pool" by combining 100 µl from each of the wells of the micro-titer plate. The culture was diluted appropriately in BHI to doses of approximately 10⁴, 10⁵, 10⁶ CFU/ml and 0.2 ml of each dilution was used to infect female 14-16 g BALB/c mice by intraperitoneal administration. At two days post-infection, one or two surviving mice were euthanized and the spleens harvested. The entire spleen was homogenized

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in 1.0 ml sterile 0.9 % saline. Dilutions of the homogenate from 10⁻² to 10⁻⁵ were prepared and plated onto BHIN⁵⁰K⁵⁰ plates. Following overnight growth, at least 20,000 colonies were pooled in 10 mls BHI broth to form the "recovered pool" and 0.5 ml of the recovered pool was centrifuged at 3,500 X g and the pellet used to prepare genomic DNA according to a previously described protocol [Wilson, *In* F. M. Ausubel, *et al.*,(ed.), <u>Current Protocols in Molecular Biology</u>, vol. 1. John Wiley and Sons, New York, p. 2.4.1-2.4.5. (1997)].

Initial experiments with virulent wild-type *P. multocida* indicated that organisms could be recovered from the spleen, lungs, kidneys, and liver indicating a truly septicemic model of infection. Dot blots for both the "input" and "recovered" pools were performed as described in Example 1 and evaluated both by visual inspection and by semi-quantitative analysis. Hybridization was carried out as described in Example 1 except that 5 µg of genomic DNA from input and recovered pools was used as template. Semi-quantitative analysis indicates whether a significant reduction in a single clone has occurred. If a mutant is unable to survive within the host, then the recovered signal should be very low compared to the input signal yielding a high input/recovered ratio. Most mutants will grow as well *in vivo* as *in vitro* and therefore a ratio of their signals should be approximately equal to 1. Clones selected by quantitative analysis as being highly reduced in the recovered pool were selected for further study. Additional clones with questionable input/recovered ratios were also selected after visually evaluating films made from the dot blots.

Example 3 Determination of Virulence for *P. multocida* Candidate Mutants

Each potential mutant which exhibited reduced recovery from splenic tissue was isolated from the original pool plate and used individually in a challenge experiment to verify and roughly estimate the attenuation caused by the transposon mutation. Individual candidate mutants from *in vivo* screens were grown on Sheep Blood Agar plates overnight in 5% CO₂ at 37°C. Approximately six colonies of each mutant were inoculated into BHI broth and allowed to grow for six hours. Dilutions were prepared and five mice each were infected as described above with 10², 10³, 10⁴

and 10^5 CFU each. Attenuation was determined by comparing mortality after six days relative to the wild type. Surviving mice were presumed to be protected and then challenged with a dose of wild type P. multocida at a concentration approximately 200-fold greater than the LD_{50} for the wild type strain. Survival rate was then determined for each challenged group of mice.

Results indicated that 62 of 120 potential transposon mutants were attenuated, having an approximate LD_{50} of at least 10 fold higher than the wild type strain. The clones and their approximate LD_{50} values are listed in Table 1. A control experiment with the wild type strain was run in parallel with each set of challenges and in all cases mortality in wild type-challenged groups was 100%.

In addition to LD_{50} values, Table 1 also provides data from vaccination and challenge experiments. Briefly, groups of mice (n = 5 to 10) were vaccinated by intraperitoneal injection with the individual P. multocida strains shown in Table 1 at a dose that was approximately 200 times greater than the LD_{50} of the virulent, wild type strain. Animals were observed for 28 days after which mortality figures were calculated.

Table 1

Table 1
P. multocida Virulence Genes

Nucleotide SEQ ID NO:	Representative Isolate	PossibleGene Function	Vaccination # survivors/total	Challenge # survivors/total	LD ₅₀	
SEQ ID NO.	Isolate	, ranction	" Sui VIVOI S/total	# survivors/total		
_	wild type	-	0/10	-	<10	
23	PM1B1	guaB	10/10, 10/10, 10/10	9/10, 9/10	4.3 x 106	
11	PMIDI	dsbB	10/10, 5/10	10/10, 5/5	8.4 x 104	
3	PM1BD7	atpG	5/5, 10/10	10/10	>3 x 105	
74	PM1BE11	yhcJ (HI0145)	10/10	5/10	>2 x 105	
70	PM1BF6	yabK	3/5, 8/10	9/9	>2 x 105	
		(HI1020)				
19	PM2G8	fhaC	4/5, 9/10	9/9	>4 x 105	
76	PM3C9	yiaO	3/5		>6 x 105	
		(HI0146)				
118	PM3G11	UnkO	4/5, 10/10	10/10	>3 x 105	
31	PM7B4	iroA (UnkB)	0/5			
17	PM4C6	fhaB (fhaB2)	2/5, 10/10, 9/10	10/10, 9/9	>3 x 106	
9	PM4G10-T9	dnaA	4/5		>5 x 105	
1	PM4D5-T5	atpB	5/5		>4 x 105	
53	PM4D5-T1	UnkC2	5/5		>4 x 105	
15	PM4F2	fhaB (fhaB1)	3/5, 6/10, 10/10	6/6, 10/10	>3 x 105	
41	PM5F7	mreB	4/5		1 x 103	
7	PM5E2	devB	0/5, 3/10	2/3	ND	
68	PM6H5-T1	xylA	5/5		>3 x 105	
78	PM6H8	yigF (HI0719)	5/5, 9/10	9/9	>3 x 105	
108	PM7D12	pnp	5/5, 9/10	9/9		
51	PM8C1R1-T2	UnkC1	5/5		~6 x 105	

Nucleotide	Representative	PossibleGene	Vaccination	Challenge	LD ₅₀
SEQ ID NO:	Isolate	Function	# survivors/total	# survivors/total	
37	PM8C1-T3	mglB	5/5		~6 x 105
58	PM8C1R1-T6	UnkD1	5/5		~6 x 105
45	PM10H7	purF (H11207)	3/5, 8/10, 8/10	8/8, 8/8	>3 x 105
25	PM10H10-T2	HII 501	5/5		>1 x 104
72	PM11G8-T2	ygiK	5/5		>2.4 x 10
21	PM11G8-T4	greA	5/5		>2.4 x 10
84	PM12H6	yyam (HI0687)	3/5, 0/10		~2.2 x 10
33	PM15G8-T2	kdtB	5/5		>1.2 x 10
116	PM15G8-T1	UnkK	5/5		>1.2 x 10
104	PM16G11-T1	hmbR	3/5		>1.9 x 10
29	PM16G11-T2	hxuC	3/5		>1.9 x 10
35	PM16H8	lgtC	5/5, 10/10	10/10	>2.4 x 10
80	PM16H3	yleA (HI0019)	5/5, 10/10		> 2.0 x 10
49	PM17H6-T1	sopE	4/5		-6 x 105
120	PM17H6	UnkP	4/5		~6 x 105
5	PM18F5-T8	cap5E	5/5		>2.4 x 10
82	PM18F5-T10	yojB (Hl0345)	5/5		>2.4 _. x 10
13	PM19A1	exbB	5/5, 10/10	10/10	>1.2 x 10
112	PM19D4	rcí	5/5, 8/10	8/8	~1.6 x 10
39	PM20A12	mioC (HI0669)	3/5, 8/10	8/8	-2 x 104
60	PM20C2	UnkD2	5/5, 10/10	10/10	>8.2 x 10

Example 4
Cloning and Identification of Genes Required for *P. multocida* Virulence

Each transposon mutant which was verified to be attenuated was analyzed further to determine the identity of the disrupted open reading frame. DNA from each mutant was amplified, purified, and digested with restriction enzymes that were known not to cut within the transposon and generally produced 4-8 kb fragments that hybridized with the transposon. Using selection for kanamycin resistance encoded by the transposon, at least one fragment for each transposon mutant was cloned.

Southern hybridization with multiple restriction enzymes was performed for each attenuated mutant using a labeled 1.8 kb *MluI* fragment from pLOF/Km as a probe to identify a suitably sized fragment for cloning. The mini-Tn10 element and flanking DNA from each mutant was cloned into pUC19 and the flanking sequence determined using internal primers TEF32 and TEF40, primer walking and in some cases universal pUC-19 primers.

TEF-32 GGCAGAGCATTACGCTGAC SEQ ID NO: 95
TEF-40 GTACCGGCCAGGCGGCCACGCGTATTC SEQ ID NO:96

Sequencing reactions were performed using the BigDye™ Dye Terminator Chemistry kit from PE Applied Biosystems (Foster City, CA) and run on an ABI Prism 377 DNA Sequencer. Double stranded sequence for putative interrupted open reading frames was obtained for each clone. Sequencer 3.0 software (Genecodes, Corp., Ann Arbor, MI) was used to assemble and analyze sequence data. GCG programs [Devereux, et al., 1997. Wisconsin Package Version 9.0, 9.0 ed. Genetics Computer Group, Inc., Madison] were used to search for homologous sequences in currently available databases.

In 37% of the clones that were identified as being attenuated, there were multiple insertions of the mini-Tn10 transposable element. Each insertion including its flanking sequence was cloned individually into pGP704 and mated into the wild-type strain to produce new mutants of *P. multocida*, each carrying only one of the multiple original insertions. Individual mutants were retested individually to determine the insertion responsible for the attenuated phenotype. The nucleotide sequence of the disrupted, predicted open reading frame was determined by sequencing both strands, and the predicted amino acid sequence was used to search currently available databases for similar sequences. Sequences either matched known genes, unknown genes, and hypothetical open reading frames previously sequenced or did not match any previously identified sequence. For those genes having homology to previously identified sequences, potential functions were assigned as set out in Table 1.

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Example 5 Identification of Related Genes in Other Species

In separate experiments, STM was also performed using *Actinobacillus* pleuropneumoniae (App). One of the App strains contained an insertion in a gene that was sequenced (SEQ ID NO: 97) and identified as a species homolog of the *P. multocida* atpG gene. This result suggested the presence in other bacterial species of

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homologs to previously unknown *P. multocida* genes that can also be mutated to produce attenuated strains of the other bacterial species for use in vaccine compositions. In order to determine if homologs of other *P. multocida* genes exists in other bacterial species, Southern hybridization was performed on genomic DNA from other species using the *A. pleuropneumoniae atpG* gene as a probe.

Actinobacillus pleuropneumoniae, Pasteurella haemolytica (Ph), P. multocida, and Haemophilus somnus (Hs) genomic DNA was isolated using the CTAB method and digested with EcoRI and HindIII for two hours at 37°C. Digested DNA was separated on a 0.7% agarose gel at 40V in TAE buffer overnight. The gel was immersed sequentially in 0.1 M HCL for 30 minutes, twice in 0.5 M NaOH/1.5 M NaCl for 15 minutes each, and twice in 2.5 M NaCl/1 M Tris, pH 7.5. The DNA was transferred to nitrocellulose membranes (Amersham Hybond N⁺) overnight using 20X SSC buffer (3 M NaCl/0.3 M sodium citrate). The DNA was crosslinked to the membrane using a UV Stratalinker on autocrosslink setting (120 millijoules). The membrane was prehybridized in 5X SSC/ 1% blocking solution/0.1% sodium lauroyl sarcosine/0.02% SDS at 50°C for approximately seven hours and hybridized overnight at 50°C in the same solution containing a PCR generated atgG probe.

The probe was prepared using primers DEL-1389 (SEQ ID NO: 98) and TEF-46 (SEQ ID NO: 99) in a with a GeneAmp XL PCR kit in a GeneAmp PCR System 2400. Template was genomic A. pleuropneumoniae DNA.

DEL-1389 TCTCCATTCCCTTGCTGCGGCAGGG SEQ ID NO: 98
TEF-46 GGAATTACAGCCGGATCCGGG SEQ ID NO: 99

The PCR was performed with an initial heating step at 94°C for five minutes, 30 cycles of denaturation t 94°C for 30 sec, annealing at 50°C for 30 sec, and elongation at 72°C for three minutes, and a final extension step at 72°C for five minutes. The amplification products were separated on an agarose gel, purified using a QIAquick gel purification kit (QIAGEN), and labeled using a DIG-High Primer kit (Boehringer Mannheim). The blot was removed from the hybridization solution and rinsed in 2X

SSC and washed two times for five minutes each wash in the same buffer. The blot was then washed two times for 15 minutes each in 0.5X SSC at 60°C. Homologous bands were visualized using a DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

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Single bands were detected in *Pasteurella haemolytica*, *Haemophilus* somnus and A. pleuropneumoniae using EcoRI digested DNA. Two bands were detected using EcoRi digested DNA from *Pasteurella multocida*.

Example 6 Construction of a Library of Tagged-Transposon P. multocida Mutants

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Transposon mutagenesis using pLOF/Km has previously been reported to be functional and random in *A. pleuropneumoniae* [Tascon, *et al.*, *J Bacteriol*. 175:5717-22 (1993)]. To construct tagged transposon mutants of *A. pleuropneumoniae*, each of 96 *E. coli* S17-1:λpir transformants containing preselected tagged plasmids (pTEF-1:[NK]₃₅) was used in conjugative matings to generate transposon mutants of *A. pleuropneumoniae* strain AP225, a serotype 1 spontaneous nalidixic acid resistant mutant derived from an in vivo passaged ATCC 27088 strain. *A. pleuropneumoniae* strains were grown on Brain Heart Infusion (BHI) (Difco Laboratories, Detroit, MI) media with 10 μg/ml B-nicotinamide adenine dinucleotide (V¹⁰), (Sigma, St. Louis, Missouri) at 37°C and in 5% CO₂ when grown on plates. *E. coli* S17-1:λpir (λpir, *recA*, *thi*, *pro*, *hsdR*(r_k-,m_k+), RP4-2, (Tc^R::Mu), (Km^R::Tn7), [Tmp^R], [Sm^R]) was propagated at 37°C in Luria-Bertani (LB) medium. Antibiotics when necessary were used at 100 μg/ml ampicillin (Sigma), 50 μg/ml nalidixic acid (N⁵⁰)(Sigma), and 50 (K⁵⁰) or 100 (K¹⁰⁰) μg/ml of kanamycin (Sigma).

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Matings were set up by growing each *E. coli* S17-1:λpir/pTEF1:[NK]₃₅ clone and the AP225 strain to late log phase. A 50 μl aliquot of culture for each tagged-pTEF-1 clone was mixed with 150 μl of the APP225 culture, and then 50 μl of each mating mixture was spotted onto 0.22 μM filters previously placed onto BHIV¹⁰ plates containing 100 μM IPTG and 10 mM MgSO₄. Following overnight incubation at 37°C with 5% CO₂, mating mixtures were washed off of each filter into 2 ml of PBS and 200 μl of each was plated onto BHIV¹⁰N⁵⁰K¹⁰⁰ plates. After selective

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overnight growth, colonies were assembled into microtiter plates by toothpick transfer into 200 μl BHIV ¹⁰N ⁵⁰K ⁵⁰ making sure that each well in a microtiter plate always contained a transposon mutant with the same sequence tag. Following overnight growth, 50 μl of 75% glycerol was added to each well and plates were stored frozen at -80°C.

APP does not appear to have as much bias towards multiple insertions of the mini-Tn10 element as did P. multocida. Only approximately 3% of the mutants were determined to contain multiple insertions, which is in agreement with the 4% previously reported [Tascon, et al., J Bacteriol. 175:5717-22 (1993)]. A problem in APP consisted of identifying numerous mutants (discussed below) containing insertions into 23S RNA regions: 28 total mutants with insertions into 13 unique sites. This may indicate that 23S RNA contains preferential insertion sites and that the growth of APP is affected by these insertions enough to result in differential survival within the host. Southern blot analysis using an APP 23S RNA probe suggests that APP may contain only three ribosomal operons as compared to five in H. influenzae [Fleischmann, et al., Science 269:496-512 (1995)] and seven complete operons in E. coli [Blattner, et al., Science 277:1453-1474 (1997)]. This site preference and its effect on growth rate may be a significant barrier to "saturation mutagenesis" since a significant number of clones will contain insertions into these rRNAs and large volume screening will be necessary to obtain additional unique attenuating mutations.

Example 7 Porcine Screening for Attenuated A. pleuropneumoniae Mutants

Twenty pools of A. pleuropneumoniae transposon mutants, containing a total of approximately 800 mutants, were screened using a porcine intratracheal infection model. Each pool was screened in two separate animals.

Frozen plates of pooled *A. pleuropneumoniae* transposon mutants were removed from -80°C storage and subcultured by transferring 20 µl from each well to a new 96 well round bottom plate (Corning Costar, Cambridge, MA, USA) containing 180 µl of BHIV¹⁰N⁵⁰K⁵⁰. Plates were incubated without shaking overnight at 37°C in

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5% CO₂. Overnight plates were then subcultured by transferring 10 µl from each well to a new flat bottomed 96 well plate (Corning Costar) containing 100 µl of BHIV10 per well and incubating at 37°C with shaking at 150 rpm. The OD₅₆₂ was monitored using a microtiter plate reader. At an OD_{562} of approximately 0.2 to 0.25, each plate was pooled to form the "input pool" by combining 100 µl from each of the wells of the microtiter plate. The culture was diluted appropriately in BHI to approximately 2 X 10⁶ CFU/ml. For each diluted pool, 4.0 ml was used to infect 10-20 kg SPF pigs (Whiteshire-Hamroc, Albion, IN) by intratracheal administration using a tracheal tube. At approximately 20 hours post-infection, all surviving animals were euthanized and the lungs removed. Lavage was performed to recover surviving bacteria by infusing 150 mls of sterile PBS into the lungs, which were then massaged to distribute the fluid. The lavage fluid was recovered, and the process was repeated a second time. The lavage fluid was centrifuged at 450 x g for 10 minutes to separate out large debris. Supernatants were then centrifuged at 2,800 x g to pellet the bacteria. Pellets were resuspended in 5 mls BHI and plated in dilutions ranging from 10⁻² to 10⁻⁵ onto BHIV¹⁰N⁵⁰K⁵⁰ plates. Following overnight growth, at least 100,000 colonies were pooled in 10 mls BHI broth to form the "recovered pools". A 0.7 ml portion of each recovered pool was used to prepare genomic DNA by the CTAB method [Wilson, In Ausubel, et al., (eds.), Current Protocols in Molecular Biology, vol. 1. John Wiley and Sons, New York, p. 2.4.1-2.4.5 (1997)].

Recovery from the animals routinely was in the $10^8\,\mathrm{CFU}$ range from lung lavage.

Dot blots were performed and evaluated both by visual inspection and by semi-quantitative analysis as described previously. All hybridizations and detections were performed as described. Briefly, probes were prepared by a primary PCR amplification, followed by agarose gel purification of the desired product and secondary PCR amplification incorporating dig-dUTP. Oligonucleotides including TEF5, TEF6, TEF24, TEF25, TEF48 and TEF62, were synthesized by Genosys Biotechnologies (The Woodlands, TX). Primers TEF69, TEF65, and TEF66 were also used for inverse PCR reactions and sequencing.

TEF69	GACGTTTCCCGTTGAATATGGCTC	SEQ ID NO: 166
TEF65	GCCGGATCCGGGATCATATGACAAGA	SEQ ID NO: 167
TEF66	GACAAGATGTGTATCCACCTTAAC	SEQ ID NO: 168

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The labeled PCR product was then digested with *HindIII* to separate the constant primer arms from the unique tag region. The region containing the labeled variable tag was excised and the entire gel slice was then dissolved and denatured in DIG EasyHyb. Dot blots were prepared and detected using the standard CSPD detection protocol. Film exposures were made for visual evaluation, and luminescent counts per second (LCPS) were determined for each dot blot sample. The LCPS_{input} / LCPS recovered</sub> ratio for each mutant was used to determine mutants likely to be attenuated.

Clones selected as being present in the input pool but highly reduced in the recovered pool were selected for further study. Additional clones with questionable input/recovered ratios were also selected after visually evaluating films made from the dot blots. A total of 110 clones were selected.

Example 8 Identification of A. pleuropneumoniae Virulence Genes

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A partial flanking sequence was determined for each of the 110 mutants by inverse PCR and direct product sequencing. Inverse PCR was used to generate flanking DNA products for direct sequencing as described above.

Sequencing reactions were performed using the BigDyetm Dye Terminator Chemistry kit from PE Applied Biosystems (Foster City, CA) and run on an ABI Prism 377 DNA Sequencer. Sequencher 3.0 software (Genecodes, Corp., Ann Arbor, MI) was used to assemble and analyze sequence data. GCG programs [Devereux and Haeberli, Wisconsin Package Version 9.0, 9.0 ed. Genetics Computer Group, Inc., Madison (1997)] were used to search for homologous sequences in currently available databases.

Table 2 shows the *A. pleuropneumoniae* genes identified and extent to which open reading frames were determinable. Sequence identification numbers are provided for nucleotide sequences as well as deduced amino acid sequences where located.

Table 2

A. pleuropneumoniae Open Reading Frames

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	Complete Open Readi	ing Frame	NO Start Codon - Stop Codon				
	atpH	SEQ ID NO: 134	dksA	SEQ ID NO: 136			
10	aptG	SEQ ID NO: 132	dnaK	SEQ ID NO: 138			
	exbB	SEQ ID NO: 140	HI0379	SEQ ID NO: 144			
	OmpP5	SEQ ID NO: 152					
	OmpP5-2	SEQ ID NO: 150	NO Start Codon - NO	Stop Codon			
	tig	SEQ ID NO: 160	pnp	SEQ ID NO: 154			
15	fkpA	SEQ ID NO: 142	apvA-or 1	SEQ ID NO: 122			
	hupA	SEQ ID NO: 146	apvA-or 2	SEQ ID NO: 124			
	rpmF	SEQ ID NO: 158	apvB	SEQ ID NO: 126			
	-		apvD	SEQ ID NO: 130			
	Start Codon - NO Stor	o Codon					
20	lpdA	SEQ ID NO: 148	RNA or Noncoding Se	equences			
	potD	SEQ ID NO: 156	tRNA-leu	SEQ ID NO: 162			
	yaeE	SEQ ID NO: 164	tRNA-glu	SEQ ID NO: 163			
	apvC	SEQ ID NO: 128					

The putative identities listed in Table 3 (below, Example 9) were assigned by comparison with bacterial databases. The 110 mutants represented 35 groups of unique transposon insertions. The number of different mutations per loci varied, with some clones always containing an insertion at a single site within an ORF to clones containing insertions within different sites of the same ORF. Three multiple insertions were detected in the 110 mutants screened as determined by production of multiple PCR bands and generation of multiple sequence electropherograms.

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Example 9 Competition Challenge of A. pleuropneumoniae Mutants with Wild Type APP225

A representative clone from each of the unique attenuated mutant groups identified above that was absent or highly reduced in the recovered population was isolated from the original pool plate and used in a competition challenge experiment with the wild type strain (AP225) to verify the relative attenuation caused by the transposon mutation. Mutant and wild type strains were grown in BHIV¹⁰ to an OD₅₉₀ of 0.6 – 0.9. Approximately 5.0 x 10⁶ CFU each of the wild type and mutant strains were added to 4 mls BHI. The total 4 ml dose was used infect a 10-20 kg SPF pig by intratracheal administration with a tracheal tube. At approximately 20 hours post-infection, all surviving animals were enthanized and the lungs removed. Lung lavages were performed as described above. Plate counts were carried out on BHIV¹⁰N⁵⁰ and BHIV¹⁰N⁵⁰K¹⁰⁰ to determine the relative numbers of wild type to mutant in both the input cultures and in the lung lavage samples. A Competitive Index (CI) was calculated as the [mutant CFU / wild type CFU]_{input} / [mutant CFU / wild type CFU]_{recovered}.

Of the 35 potential transposon mutants, 22 were significantly attenuated, having a competitive index (CI) of less than 0.2. A transposon mutant that did not seem to be attenuated based on the STM screening results was chosen from one of the pools as a positive control. This mutant had a CI in vivo of approximately 0.6. An in vitro competition was also done for this mutant resulting in a CI of 0.8. The mutant was subsequently determined to contain an insertion between 2 phenylalanine tRNA's.

Competitive indices for unique attenuated single-insertion mutants are listed in Table 3. Competitive indices for *atpG*, *pnp*, and *exbB* App mutants indicated that the mutants were unable to compete effectively with the wild type strains and were therefore attenuated.

Table 3
Virulence and Proposed Function of A. pleuropneumoniae Mutants

Mutant	Similarity	Putative or Known Functions	C.I.
AP20A6	atpH	ATP synthase	.009
AP7F10	atpG_	ATP synthase	.013
AP17C6	lpdA	dihydrolipoamide dehydrogenase	.039
AP11E7	exbB	transport of iron compounds	.003,.003,.00
AP3H7	potD	Spermidine/putrescine transport	.308
AP8H6	OmpP5	Adhesin / OmpA homolog	.184
AP18H8	OmpP5-2	Adhesin / OmpA homolog	.552
AP13E9	tig	Peptidyl-prolyl isomerase	.050
AP13C2	fkpA	Peptidyl-prolyl isomerase	<.001
AP15C11	рпр	Polynucleotide phosphorylase	.032
AP18F12	hupA	Histone – like protein	.001
AP20F8	dksA	Dosage dependent suppressor of dnaK mutations	.075
AP5G4	dnaK	Heat shock protein - molecular chaperone	.376
AP17C9	tRNA-leu	Protein Synthesis	.059
AP5D6	tRNA-glu	Protein Synthesis	.055
AP18B2	rpmF	Protein Synthesis	.112
AP10E7	yaeA	Unknown	.001
AP19A5	HI0379	Unknown	.061
AP10C10	арvА	Unknown	.157
AP18F5	арvВ	Unknown	.103
AP2A6	арvС	Unknown .	.091
AP2C11	apvD	Unknown	.014

Accuracy of the CI appeared to be very good as the *exbB* mutant was competed within three different animals yielding CI's of 0.003, 0.003 and 0.006. The use of a Competitive Index number to assign attenuation based upon one competition in a large animal study was further confirmed based on preliminary vaccination results in pigs with 7 mutants (n=8) described below in Example 11.

Example 10 Characterization of Attenuated A. pleuropneumoniae Virulence Genes

The A. pleuropneumoniae genes identified represent four broad functional classes: biosynthetic enzymes, cellular transport components, cellular regulation components and unknowns.

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The atpG gene, encoding the F1- γ subunit of the F₀F₁ H+-ATPase complex, can function in production of ATP or in the transport of protons by hydrolyzing ATP. A related atpG attenuated mutant was also identified in P. multocida. Another atp gene, atpH, that encodes the F₁ δ subunit was also identified. Phenotypes of atp mutants include non-adaptable acid-sensitivity phenotype [Foster, J Bacteriol. 173:6896-6902 (1991)], loss of virulence in Salmonella typhimurium [Garcia del Portillo, et al., Infect Immun. 61:4489-4492 (1993)] and P. multocida (above) and a reduction in both transformation frequencies and induction of competence regulatory genes in Haemophilus influenzae Rd [Gwinn, et al., J Bacteriol. 179:7315-20 (1997)].

LpdA is a dihydrolipoamide dehydrogenase that is a component of two enzymatic complexes: pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase. While the relationship to virulence is unknown, production of LpdA is induced in Salmonella typhimurium when exposed to a bactericidal protein from human which may suggest that this induction may be involved in attempts to repair the outer membrane [Qi, et al., Mol Microbiol. 17:523-31 (1995)].

Transport of scarce compounds necessary for growth and survival are critical in vivo. ExbB is a part of the TonB transport complex [Hantke, and Zimmerman, *Microbiology Letters. 49*:31-35 (1981)], interacting with TonB in at least two distinct ways [Karlsson, et al., Mol Microbiol. 8:389-96 (1993), Karlsson, et al., Mol Microbiol. 8:379-88 (1993)]. Iron acquisition is essential for pathogens. In this work, attenuated exbB mutants in both APP and P. multocida have been identified. Several TonB-dependent iron receptors have been identified in other bacteria [Biswas, et al., Mol. Microbiol. 24:169-179 (1997), Braun, FEMS Microbiol Rev. 16:295-307 (1995), Elkins, et al., Infect Immun. 66:151-160 (1998), Occhino, et

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al., Mol Microbiol. 29:1493-507 (1998), Stojiljkovic and Srinivasan, J Bacteriol. 179:805-12 (1997)]. A. pleuropneumoniae produces 2 transferrin-binding proteins, which likely depend on the ExbB/ExbD/TonB system, for acquisition of iron. PotD is a periplasmic binding protein that is required for spermidine (a polyamine) transport [Kashiwagi, et al., J Biol Chem. 268:19358-63 (1993)]. Another member of the Pasteurellaceae family, Pasteurella haemolytica, contains a homologue of potD (Lpp38) that is a major immunogen in convalescent or outer membrane protein vaccinated calves [Pandher and Murphy, Vet Microbiol. 51:331-41 (1996)]. In P. haemolytica, PotD appeared to be associated with both the inner and outer membranes. The role of PotD in virulence or in relationship to protective antibodies is unknown although previous work has shown potD mutants of Streptococcus pneumoniae to be attenuated [Polissi, et al., Infect. Immun. 66:5620-9 (1998)].

Relatively few "classical virulence factors," such as adhesins or toxins with the exception of homologues to OMP P5 of Haemophilus influenzae, were identified. H. influenzae OMP P5 is a major outer membrane protein that is related to the OmpA porin family of proteins [Munson, et al., M Infect Immun, 61:4017-20] (1993)]. OMP P5 in nontypeable Haemophilus influenzae has been shown to encode a fimbrial subunit protein expressed as a filamentous structure [Sirakova, et al., Infect Immun. 62:2002-20 (1994)] that contributes to virulence and binding of both mucin and epithelial cells [Miyamoto and Bakaletz, Microb Pathog. 21:343-56 (1996), Reddy, et al., Infect Immun. 64:1477-9 (1996), Sirakova, et al., Infect Immun. 62:2002-20 (1994)]. A significant finding was identification of two distinct ORF's . that appear to encode OMP P5 homologues. This is also the case with two very similar proteins, MOMP and OmpA2 from Haemophilus ducreyi. It remains to be determined whether both are functionally involved in the production of fimbriae and whether the presence of two such ORFs represents a divergent duplication with redundant or complementing functions. Interestingly, the two OMP P5 mutants seem to have disparate CI values, suggesting a difference in essentiality or functionality for only one copy. OMP P5 has been shown to undergo molecular variation during chronic infections [Duim, et al., Infect Immun. 65:1351-1356 (1997)], however, this

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appears to be restricted to a single gene undergoing point mutations resulting in amino acid changes rather than "type switching" due to differential expression of multiple genes.

Protein folding enzymes are important accessories for the efficient folding of periplasmic and extracellular proteins, and two genes were identified whose products have peptidyl-prolyl isomerase activity: fkpA and tig (trigger factor). FkpA is a periplasmic protein that is a member of the FK506-binding protein family [Horne and Young, Arch Microbiol. 163:357-65 (1995); Missiakas, et al., Mol Microbiol. 21:871-84 (1996)]. FkpA has been shown to contribute to intracellular survival of Salmonella typhimurium [Horne, et al., Infect Immun. 65:806-10 (1997)] and a Legionella pneumophila homolog, mip [Engleberg, et al., Infect Immun. 57:1263-1270 (1989)], is responsible for virulence and infection of macrophages [Cianciotto, et al., J. Infect. Dis. 162:121-6 (1990); Cianciotto, et al., Infect. Immun. 57:1255-1262 (1989)]. Tig, or trigger factor [Crooke and Wickner, Proc. Natl. Acad. Sci. USA. 84:5216-20 (1987), Guthrie, and Wickner, J Bacteriol. 172:5555-62 (1990), reviewed in Hesterkamp, and Bukau., FEBS Lett. 389:32-4 (1996)], is a peptidyl prolyl isomerase containing a typical FKBP region [Callebaut and Mornon, FEBS Lett. 374:211-215 (1995)], but is unaffected by FK506 [Stoller, et al., EMBO J. 14:4939-48 (1995)]. Tig has been shown to associate with the ribosomes and nascent polypeptide chains [Hesterkamp, et al., Proc Natl Acad Sci USA 93:4437-41 (1996), Stoller, et al., EMBO J. 14:4939-48 (1995)]. Possible roles include an unknown influence on cell division [Guthrie, and Wickner, J Bacteriol. 172:5555-62 (1990)] in E. coli, a role in the secretion and activation of the Streptococcus pyogenes cysteine proteinase [Lyon, et al., EMBO J. 17:6263-75 (1998)] and survival under starvation conditions in Bacillus subtilis [Gothel, et al., Biochemistry 37:13392-9 (1998)].

Bacterial pathogens employ many mechanisms to coordinately regulate gene expression in order to survive a wide variety of environmental conditions within the host. Differences in mRNA stability can modulate gene expression in prokaryotes [Belasco and Higgins, Gene 72:15-23 (1988)]. For example, rnr (vacB) is required for expression of plasmid borne virulence genes in Shigella flexneri [Tobe, et al., J

Bacteriol. 174:6359-67 (1992)] and encodes the RnaseR ribonuclease [Cheng, et al., J. Biol. Chem. 273:14077-14080 (1998)]. PNP is a polynucleotide phosphorylase that is involved in the degradation of mRNA. Null pnp/rnr mutants are lethal, suggesting a probable overlap of function. It therefore is possible that both rnr and pnp are involved in the regulation of virulence gene expression. A pnp mutant of P. multocida is avirulent in a mouse septicemic model (Example 2)]. Other pnp-associated phenotypes include competence deficiency and cold sensitivity in Bacillus subtilis [Wang and Bechhofer, J Bacteriol. 178:2375-82 (1996)].

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HupA is a bacterial histone-like protein, which in combination with HupB constitute the HU protein in E. coli. Reports have suggested that hupA and hupB single mutants do not demonstrate any observable phenotype [Huisman, et al., J Bacteriol. 171:3704-12 (1989), Wada, et al., J Mol Biol. 204:581-91 (1988)], however, hupA-hupB double mutants have been shown to be cold sensitive, sensitive to heat shock and blocked in many forms of site-specific DNA recombination [Wada, et al., J Mol Biol. 204:581-91 (1988), Wada, et al., Gene. 76:345-52 (1989)]. One limited data previously indicated that hupA is directly involved in virulence [Turner, et al., Infect Immun. 66:2099-106 (1998)]. The mechanism of hupA attenuation remains unknown.

DnaK is a well known and highly conserved heat shock protein involved in regulatory responses to various stressful environmental changes [reviewed in Lindquist and Craig, Annu Rev Genet. 22:631-77 (1988)]. DnaK is also one of the most significantly induced stress proteins in Yersinia enterocolitica after being phagocytosed by macrophages [Yamamoto, et al., Microbiol Immunol. 38:295-300 (1994)] and a Brucella suis dnaK mutant failed to multiply within human macrophage-like cells [Kohler, et al., Mol Microbiol. 20:701-12 (1996)]. In contrast, another intracellular pathogen, Listeria monocytogenes, did not show induction of dnaK after phagocytosis [Hanawa, et al., Infect Immun. 63:4595-9 (1995)]. A dnaK mutant of Vibrio cholera affected the production of ToxR and its regulated virulence factors in vitro but similar results were not obtained from in vivo grown cells [Chakrabarti, et al., Infect Immun. 67:1025-1033 (1999)]. The CI of A.

pleuropneumonia dnaK mutant was higher than most of the attenuated mutants although still approximately half of the positive control strain.

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DksA is a dosage dependent suppressor of filamentous and temperature-sensitive growth in a dnaK mutant of E. coli [Kang and Craig, J Bacteriol. 172:2055-64 (1990)]. There is currently no defined molecular function for DksA, but the gene has been identified as being critical for the virulence of Salmonella typhimurium in chickens and newly hatched chicks [Turner, et al., Infect Immun. 66:2099-106 (1998)]. In that work, it was noted that the dksA mutant did not grow well with glucose or histidine but did grow well with glutamine or glutamate as the sole carbon source. This observation may indicate that the dksA mutant is somehow impaired in the biosynthesis of glutamate [Turner, et al., Infect Immun. 66:2099-106 (1998)].

Three genes were identified that have roles in protein synthesis: tRNA-leu, tRNA-glu and rpmF. Excluding protein synthesis, tRNA's also have a wide variety of functional roles in peptidoglycan synthesis [Stewart, et al., Nature 230:36-38 (1971)], porphyrin ring synthesis [Jahn, et al., Trends Biochem Sci. 17:215-8 (1992)], targeting of proteins for degradation [Tobias, et al., Science 254:1374-7 (1991)], post-translational addition of amino acids to proteins [Leibowitz and Soffer, B.B.R.C. 36:47-53 (1969)] and mediation of bacterial-eukaryotic interactions [Gray, et al., J Bacteriol. 174:1086-98 (1992), Hromockyj, et al., Mol Microbiol. 6:2113-24 (1992)]. More specifically, tRNA-leu is implicated in transcription attenuation [Carter, et al., Proc. Natl. Acad. Sci. USA 83:8127-8131 (1986)], lesion formation by Pseudomonas syringae [Rich and Willis, J Bacteriol. 179:2247-58 (1997)] and virulence of uropathogenic E. coli [Dobrindt, et al., FEMS Microbiol Lett. 162:135-141 (1998), Ritter, et al., Mol Microbiol. 17:109-21 (1995)]. It is unknown whether the tRNA that we have identified represents a minor species of tRNA-leu in A. pleuropneumoniae. Regardless, it is possible that tRNA-leu may have any one of a wide range of functions. RpmF is a ribosomal protein whose gene is also part of an operon containing fatty acid biosynthesis enzymes in E. coli. Further work will be required to indicate if this is the case in A. pleuropneumoniae, although the same

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clustering of fab genes and rpmF occurs in Haemophilus influenzae [Fleischmann, et al., Science 269:496-512 (1995)]. The expression of the fab genes is not necessarily dependent on transcripts originating upstream of rpmF as there has been a secondary promoter identified within rpmF [Zhang and Cronan, Jr., J Bacteriol. 180:3295-303 (1998)].

The final class of attenuated mutants includes mutations within genes of unknown function or genes that have not been previously identified. Homologs of yaeA and HI0379 have previously been identified in Escherichia coli [Blattner, et al., Science 277:1453-1474 (1997)] and Haemophilus influenzae [Fleischmann, et al., Science 269:496-512 (1995)], respectively. The remaining unknowns have been designated Actinobacillus pleuropneumoniae virulence genes (apv). The apvC gene shows significant similarity to HI0893, however, the proposed similarity of HI0893 as a transcriptional repressor similar to the fatty acid response regulator Bm3R1 [Palmer, J Biol Chem. 273:18109-16 (1998)] is doubtful. The apvD gene is also most similar to a putative membrane protein (b0878) with unknown function from E. coli [Blattner, et al., Science 277:1453-1474 (1997)]. Two other unknowns, apvA and apvB had no significant matches in the public databases.

Example 11 Safety and Efficacy of A. pleuropneumoniae Mutants

Nine groups (n=8) of SPF pigs (4-5 weeks old, 3-10 kg) were used to determine the safety and efficacy of seven *A. pleuropneumoniae* mutants as live attenuated vaccine strains. Seven groups were infected intranasally with 10¹⁰ CFU of each mutant on day 1. One group was vaccinated on days 1 and 15 with the commercially available vaccine Pleuromune (Bayer), and one naive group was not vaccinated. On day 29, all groups were challenged intranaslally with 1-5 x 10⁵ CFU per pig of wild type APP225. All surviving animals were euthanized and necropsied on day 42 of the study. Results are shown in Table 4.

Table 4
Efficacy of A. pleuropneumoniae Mutants

% Mortality following intranasal

	<u>Vaccine</u>	challenge				
		Vaccination	Challenge			
5	Pleuromune	0	37.5			
	exbB	0	0			
	tig	12.5	0			
	fkpA	12.5	0			
	HI0385	50.0	0			
10	pnp	0	0			
	yaeE	0	0			
	atpG	0	0			
	None	N/A	50.0			

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The exbB, atpG, pnp, and yaeA mutants caused no mortality when administered at a dosage of 10^{10} CFU intranasally. The fkpA and tig mutant groups had one death each and the HI0379 group (highest CI of the 7 mutants tested shown in Example 9) had four deaths. Wildtype LD₅₀ using this model was generally 1 x 10^7 CFU, indicating that each of these mutants is at least 100 fold attenuated and that there is a reasonable correlation between CI and attenuation.

Example 12 Identification of *P.(Mannheimia) haemolytica* Species Homologs

Based on the sequences of virulence genes identified in *P. multocida* and *A. pleuropneumoniae*, attempt were made to identify related genes, *i.e.*, species homologs, in *P. (Mannheimia) haemolytica*. PCR was utilized with the degenerate primers shown below to attempt amplification of the *P. (Mannheimia) haemolytica* genes as indicated. Primer sequences, synthesized by Sigma-Genosys (The Woodlands, TX), include standard single letter designations, wherein B indicates

either (C,G or T), D indicates either (G,A or T), H indicates either (A,C or T), K indicates either (G or T), M indicates either (A or C), N indicates either (A,G,C or T), R indicates either (A or G), S indicates either (G or C), V indicates either (G, A, or C), W indicates either (A or T), and Y indicates either (C or T).

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	atpG	TEF146 TEF148	ATG GCN GGN GCN AAR GAR AT GCN GCY TTC ATN GCN ACC AT	SEQ ID NO: 176 SEQ ID NO: 177
10	guaB	TEF240 TEF243	GGN TTY ATY CAY AAA AAY ATG TCT TTN GTR ATN GTN ACA TCR TG	SEQ ID NO: 178 SEQ ID NO: 179
	pnp	TEF141 TEF142	GCS GGY AAA CCR CGT TGG GAT TGG CRC CTA ARA TRT CTG AAA GCA CCA C	SEQ ID NO: 180 SEQ ID NO: 181
15	purF	TEF244 TEF247	ATG TGY GGN ATY GTN GGN AT CAT ATC AAT ACC ATA CAC ATT	SEQ ID NO: 182 SEQ ID NO: 183
	yjgF	TEF162 TEF163	GGN CCN TAY GTN CAR G NGC NAC YTC NAC RCA	SEQ ID NO: 184 SEQ ID NO: 185

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For amplification of initial degenerate PCR products, a 50 μ l reaction was set up using 3.3X XL buffer II (PE Applied Biosystems), 200 μ M dNTPs, 25 pmol each of the appropriate primers, 0.8 mM MgCl₂, 0.5 U r*Tth* DNA polymerase, XL (PE Applied Biosystems) and approximately 1 μ g of TF1 DNA.

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Cycle conditions were 94°C for 1.5 min; followed by 35 cycles of 94°C for 15 s, 40-60°C for 60 s, 72°C for 1.5 min; and a final hold at 72°C for 5 min. Each PCR product was band purified from an agarose gel using the QIAGEN Gel Extraction Kit (QIAGEN, Valencia CA).

Sequencing reactions were performed using the BigDyetm Dye Terminator Chemistry kit from PE Applied Biosystems (Foster City, CA) and run on an ABI Prism 377 DNA Sequencer. Double stranded sequence for the open reading frame (ORF) for each clone was obtained. Sequencher 3.0 software (Genecodes, Corp., Ann Arbor, MI) was used to assemble and analyze sequence data. GCG programs were used to confirm the identity of the ORF by searching for homologous sequences in currently available

35 databases.

The Vectorette Kit (Genosys Biotechnologies, The Woodlands, TX) was used to obtain additional flanking sequence for each of the genes. Vectorette libraries were prepared according to the manufacturer's suggested protocol. Perkin Elmer Applied Biosystems GeneAmp XL PCR Kit components were used to create the Vectorette PCR products with the following reaction conditions. A 50 μl reaction was set up using 3.3X XL buffer II (PE Applied Biosystems), 200 μM dNTPs, 25 pmol each of the appropriate primers(shown below), 0.8 mM MgCl₂, 0.5 U r*Tth* DNA polymerase, XL (PE Applied Biosystems) and 1 μl of the appropriate vectorette library. Cycle conditions were 94°C for 1.5 min; followed by 35 cycles of 94°C for 20 s, 60°C for 45s, 72°C for 4 min; and a final hold of 72°C for 7 min. The second primer for each library was the manufacturer's vectorette primer.

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Table 5

Gene	Vectorette library	Primer(s)
atpG	BglII, HindIII	TEF217 GAAGCCGCCATACGCTCTTGGG
		SEQ ID NO: 186
	ClaI	TEF218 GTTGCTTCCTTTGCCTGCACTGG
		SEQ ID NO: 187
guaB	EcoRI	TEF265 GGCTCAGAAACAATACCACTTTCA
	1	SEQ ID NO: 188
	HindIII, TaqI	TEF268 GCACCAAAGCAGAATTTGTCC
		SEQ ID NO: 189
pnp	ClaI, HincII	TEF219 GGTGATGATGTCGATGATAGTCCC
PP		SEQ ID NO: 190
<u></u> -	TaqI,	TEF220 GGCGTATTAGCCGTGATGCCAACC
		SEQ ID NO: 191
	BamHI	TEF286 GACCACTTAGGCGATATGGACTT
		SEQ ID NO: 192
purF	TaqI	TEF271 ACCATCATAAATCGCCTGATTC
		SEQ ID NO: 193 TEF292 ACCTGCGGCATCTTGTCCTC
		SEQ ID NO: 194
	HincII	TEF274 ACGGGTTTATTTTGCCTCTG
		SEQ ID NO: 195
wie.E	ClaI	TEF221 CGCCGGTTTCAGGATTCACGGG
yjgF	Ciai	
•	EcorV	SEQ ID NO: 196 TEF281 CTGAACAACGTGAAAGCCAT
	LCOLV	
	1	SEQ ID NO: 197

Vectorette PCR products were band purified and sequenced as described above.

Polynucleotide sequences for the atpG, guaB, pnp, purF, and yjgF genes are set out in SEQ ID NOs: 166, 168, 170, 172 and 174, respectively. Polypeptides encoded by these genes are set out in SEQ ID NOs: 167, 169, 171, 173, and 175, respectively.

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Numerous modifications and variations in the invention as set forth in the above illustrative examples are expected to occur to those skilled in the art. Consequently only such limitations as appear in the appended claims should be placed on the invention.

WHAT IS CLAIMED IS:

1. A gram-negative bacteria comprising a mutation in a gene represented by a nucleotide sequence set forth in any one of SEQ ID NOs: 1, 3, 7, 9, 21, 25, 27, 29, 39, 41, 51, 53, 55, 57, 58, 60, 68, 72, 74, 76, 78, 80, 82, 84, 104, 108, 112, 116, 118, 120 122, 124, 126, 128, and 130, or species homologs thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.

- 2. The gram-negative bacteria of claim 1 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.
- 3. The gram-negative bacteria of claim 1 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.
- 4. The gram-negative bacteria of claim 1 wherein said mutation results in deletion of all or part of said gene.
- 5. An attenuated *Pasteurellaceae* bacteria comprising a mutation in a gene represented by a nucleotide sequence set forth in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172 and 174 or a species homolog thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.
- 6. The *Pasteurellaceae* bacteria of claim 5 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.
- 7. The *Pasteurellaceae* bacteria of claim 5 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.

8. The *Pasteurellaceae* bacteria of claim 5 wherein said mutation results in deletion of all or part of said gene.

- 9. The Pasteurellaceae bacteria of claim 5 selected from the group consisting of Pasteurella (Mannheimia) haemolytica, Pasteurella multocida, Actinobacillus pleuropneumoniae and Haemophilus somnus.
- 10. The *Pasteurellaceae* bacteria of claim 9 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.
- 11. The *Pasteurellaceae* bacteria of claim 9 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.
- 12. The *Pasteurellaceae* bacteria of claim 9 wherein said mutation results in deletion of all or part of said gene.
- 13. The attenuated *Pasteurellaceae* bacteria of claim 9 that is a *P. multocida* bacteria.
- 14. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.
- 15. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.
- 16. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in deletion of all or part of said gene.
- 17. The attenuated *Pasteurellaceae* bacteria of claim 9 that is a *A. pleuropneumoniae* bacteria.

18. The *Pasteurellaceae* bacteria of claim 17 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.

- 19. The *Pasteurellaceae* bacteria of claim 17 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.
- 20. The *Pasteurellaceae* bacteria of claim 17 wherein said mutation results in deletion of all or part of said gene.
- 21. An immunogenic composition comprising the bacteria according to any one of claims 1 through 20.
- 22. A vaccine composition comprising the immunogenic composition according to claim 21 and a pharmaceutically acceptable carrier.
- 23. The vaccine composition according to claim 22 further comprising an adjuvant.
- 24. A method for producing a gram-negative bacteria mutant comprising the step of introducing a mutation in a gene represented by a nucleotide sequence set forth in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174 or a species homolog thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.
- 25. A method for producing an attenuated *Pasteurellaceae* bacteria comprising the step of introducing a mutation in a gene represented by a nucleotide sequence set forth in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29,

31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172, and 174 or a species homolog thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.

- 26. A purified and isolated *Pasteurellaceae* polynucleotide comprising a nucleotide sequence selected from the group consisting of nucleotide sequences set forth in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, 164, 166, 168, 170, 172 and 174.
- 27. A purified and isolated *Pasteurellaceae* polynucleotide comprising a nucleotide sequence selected from the group consisting of nucleotide sequences set forth in SEQ ID NOs: 1, 3, 7, 9, 21, 25, 27, 29, 39, 41, 51, 53, 55, 57, 58, 60, 68, 72, 74, 76, 78, 80, 82, 84, 104, 108, 112, 116, 118, 120 122, 124, 126, 128, and 130.
- 28. A purified and isolated polynucleotide encoding a *Pasteurellaceae*. virulence gene product, or species homolog thereof, selected from the group consisting of:
 - a) the polynucleotide according to claim 27,
 - b) polynucleotides encoding a polypeptide encoded by the polynucleotide of (a), and
 - c) polynucleotides that hybridize to the complement of the polynucleotides of (a) or (b) under moderate stringency conditions.
- 29. A purified and isolated *Pasteurellaceae* polynucleotide encoding a polypeptide selected from the group consisting of polypeptides having amino acid sequences set forth in SEQ ID NOs: 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 34, 38, 40, 42, 52, 54, 56, 59, 61, 69, 71, 73, 75, 77, 79, 81, 83, 85, 101, 103, 105, 107, 109,

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- 30. The polynucleotide of claim 29 which is a DNA.
- 31. A vector comprising the DNA of claim 30.
- 32. The vector of claim 31 that is an expression vector, wherein the DNA is operatively linked to an expression control DNA sequence.
- 33. A host cell stably transformed or transfected with the DNA of claim 30 in a manner allowing the expression of the encoded polypeptide in said host cell.
- 34. A method for producing a recombinant polypeptide comprising culturing the host cell of claim 33 in a nutrient medium and isolating the encoded polypeptide from said host cell or said nutrient medium.
 - 35. A purified polypeptide produced by the method of claim 34.
- 36. A purified polypeptide comprising a polypeptide selected from the group consisting of polypeptides having amino acid sequences set forth in SEQ ID NOs: 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 34, 38, 40, 42, 52, 54, 56, 59, 61, 69, 71, 73, 75, 77, 79, 81, 83, 85, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 165, 167, 169, 171, 173, and 175.
- 37. An antibody that is specifically reactive with the polypeptide of claim 36.
 - 38. The antibody of claim 33 that is a monoclonal antibody.

39. A method of using the monoclonal antibody of claim 39 for identifying a bacteria of claim 1, 5, 9, or 13 comprising the step of contacting an extract of bacteria with said monoclonal antibody and detecting the absence of binding of said monoclonal antibody.

- 40. A method of identifying an anti-bacterial agent comprising the steps of assaying potential agents for the ability to interfere with expression or activity of gene products represented by the amino acid sequences set forth in any one of SEQ ID NOS: 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 34, 38, 40, 42, 52, 54, 56, 59, 61, 69, 71, 73, 75, 77, 79, 81, 83, 85, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 165, 167, 169, 171, 173, and 175 and identifying an agent that interferes with expression or activity of said gene products.
- 41. A method of identifying an anti-bacterial agent comprising the steps of:
 - a) measuring expression or activity of a gene product as set out in SEQ ID NOS: 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 34, 38, 40, 42, 52, 54, 56, 59, 61, 69, 71, 73, 75, 77, 79, 81, 83, 85, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 165, 167, 169, 171, 173, and 175;
 - b) contacting the gene product in (a) with a test compound
 - c) measuring expression or activity of the gene product in the presence of the test compound; and
 - d) identifying the test compound as an antibacterial agent when expression or activity of the gene product is decreased in the presence of the test compound as compared to expression or activity in the presence of the test compound.

SEQUENCE LISTING

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His Pro Phe Leu Val Asp Arg Glu Val Lys Lys Val Gly Met Ile Val 65 70 75 80

Val Ser Thr Asp Arg Gly Leu Cys Gly Gly Leu Asn Val Asn Leu Phe 85 90 95

Lys Thr Val Leu Asn Glu Met Lys Glu Trp Lys Glu Lys Asp Val Ser 100 105 110

Val Gln Leu Ser Leu Ile Gly Ser Lys Ser Ile Asn Phe Phe Gln Ser 115 120 125

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Lys Lys Gly Glu Val Asp Val Val Tyr Leu Val Tyr Asn Lys Phe Ile 165 170 175

Asn Thr Met Ser Gln Lys Pro Val Leu Glu Lys Leu Ile Pro Leu Pro 180 185 190

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Ile Tyr Glu Pro Asp Ala Lys Val Leu Leu Asp Asn Leu Leu Val Arg 210 215 220

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Glu Gln Ala Ala Arg Met Val Ala Met Lys Ala Ala Thr Asp Asn Ala 245 250 255

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Leu Ser Thr Asp Lys Ala Val Tyr Pro Ile Asn Ala Met Gly Ile 50 55 60	Ser
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Ser Ile Ile Gly Thr Arg His Gly Glu Lys Ala Phe Glu Ala Leu Leu 180 185 190

Ser Arg Glu Glu Met Val His Ala Ile Asn Glu Gly Asn Tyr Tyr Arg 195 200 205

Ile Pro Ala Asp Gln Arg Ser Leu Asn Tyr Ser Lys Tyr Val Glu Lys 210 215 220

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Arg Phe Glu Gln Ala Leu Ser Ala Val Ile Pro Gly Gln Val Phe Asp 115 120 125

Trp Ile Ile Leu Gly Met Gly Thr Asp Gly His Thr Ala Ser Leu Phe 130 140

Pro His Gln Thr Asp Phe Asp Asp Pro His Phe Ala Val Ile Ala Lys

155 145 150 160 His Pro Glu Thr Gly Gln Ile Arg Ile Ser Lys Thr Ala Lys Leu Ile Glu Gln Ala Lys Arg Val Thr Tyr Leu Val Thr Gly Ser Ser Lys Ala 180 Glu Ile Leu Lys Glu Ile Gln Thr Thr Pro Ala Glu Gln Leu Pro Tyr 20L Pro Ala Ala Lys Ile Lys Ala Lys His Gly Val Thr Glu Trp Tyr Leu Asp Lys Asp Ala Ala Lys Leu Leu 230 <210> 9 <211> 2438 <212> DNA <213> Pasteurella multocida <220> <221> CDS <222> (1635)..(2396) <220> <223> dnaA <400> 9 gacccaatgc ttgccaccgg cggctctatg attgcgacaa tcgatcttct aaaagcgaaa 60 ggctgtaaac acattaaagt gctcgtgtta gtcgccgcgc ctgaaggcat taaagcatta 120 gaagetgege accetgatat egaattatat accgeateag ttgatagtea ettaaatgaa 180 caaggctata ttattccagg tcttggtgat gccggtgata aaatttttgg cactaaataa 240 teccaacaca ageggeatet tatgeegett tttteegtte aatttatage gettacaate 300 ttaacagctt qaacactata aaatgaaaag ttaattcaga cagagagttg aaacttaaca 360 tgacaaatca aaatccccct gttcttctag aacaaaatca cgcaaaacaa gccttcgttg 420 ggctacaaat gctttttgtt gccttcggtg ctttagtcct tgttcccctg attacgggtt 480 taaatgccaa tactgcctta ttgaccgcag ggattgggac actcttattc caactttgta 540 ctggacgcca agtcccaatt ttcttagcct cttcctttgc ttttattgca ccaattcaat 600 tttattttgc cctcagtacg ttagtcaaaa ttaaaggtgc tggtgcttta caaaaagtct 720 ttccgccagt agttgttggt cccgttatta tcatcatcgg tatgggactt gcccctgttq 780 ccgtggacat ggcattaggt aaaaacagca cttatcaata taacgatgcc gtattcgttt 840 cgatggcaac attattgaca acgttaggtg ttgcggtgtt tgctaaaggc atgatgaaat 900 taatteetat catgititggt attgiegteg getatateet etgettatte tiaggettaa 960

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gat atg aaa acg tta ttt gaa gca cta agt aaa tta gat aaa gca tca Asp Met Lys Thr Leu Phe Glu Ala Leu Ser Lys Leu Asp Lys Ala Ser 225 230 235	2342
tta caa gcc caa cgt aaa tta acg att ccc ttt gta aaa gaa att tta Leu Gln Ala Gln Arg Lys Leu Thr Ile Pro Phe Val Lys Glu Ile Leu 240 245 250	2390
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Leu Leu Asn Pro Ser Phe Phe Val Tyr Pro Tyr Ser Pro Phe Phe Asp 10	
Leu Leu Asn Pro Ser Phe Phe Val Tyr Pro Tyr Ser Pro Phe Phe Asp 10 Leu Pro Phe Phe Asp 15 Phe Val Gly Cys Phe Leu Leu Glu Asn Phe Gln Leu Pro Leu Pro Ile 20 Asp Asp Glu Thr Leu Asp Asp Ash Phe Tyr Pro Asp Asn Asn 35 Leu Leu Leu Leu Asn Ser Leu Arg Lys Asn Phe Thr Cys Leu Thr Gln	
Leu Leu Asn Pro Ser Phe Phe Val Tyr Pro Tyr Ser Pro Phe Phe Asp 10	
Leu Leu Asn Pro Ser Phe Phe Val Tyr Pro Tyr Ser Pro Phe Phe Asp 15 Phe Val Gly Cys Phe Leu Leu Glu Asn Phe Gln Leu Pro Leu Pro Ile 30 His Gln Leu Asp Asp Glu Thr Leu Asp Asn Phe Tyr Pro Asp Asn Asn Asn 35 Leu Leu Leu Leu Asn Ser Leu Arg Lys Asn Phe Thr Cys Leu Thr Gln 50 Gln Phe Phe Tyr Ile Trp Gly Glu Gln Ser Ser Gly Lys Ser His Leu 65 Leu Lys Gly Ile Thr His His Phe Phe Leu Leu Gln Arg Pro Ala Ile	
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Leu Leu Asn Pro Ser Phe Phe Val Tyr Pro Tyr Ser Pro Phe Phe Asp 15 Phe Val Gly Cys Phe Leu Leu Glu Asn Phe Gln Leu Pro Leu Pro Ile 30 His Gln Leu Asp Asp Glu Thr Leu Asp Asn Phe Tyr Pro Asp Asn Asn Asn 45 Leu Leu Leu Leu Leu Asn Ser Leu Arg Lys Asn Phe Thr Cys Leu Thr Gln 60 Gln Phe Phe Tyr Ile Trp Gly Glu Gln Ser Ser Gly Lys Ser His Leu 80 Leu Lys Gly Ile Thr His His Phe Phe Leu Leu Gln Arg Pro Ala Ile 85 Tyr Val Pro Leu Glu Lys Ser Gln Tyr Phe Ser Pro Ala Val Leu Glu Asn Leu Glu Gln Gln Gln Gln Leu Val Cys Leu Asp Asn Leu Gln Ala Ile	

Pro Thr Ala Leu Pro Val Ser Leu Pro Asp Leu Ala Ser Arg Leu Arg 170 Trp Gly Glu Ser Tyr Gln Leu Val Pro Leu Asn Asp Gln Gln Lys Ile His Val Leu Gln Lys Asn Ala His Gln Arg Gly Ile Glu Leu Pro Asp Glu Val Ala Asn Phe Leu Leu Lys Arg Leu Glu Arg Asp Met Lys Thr 210 215 Leu Phe Glu Ala Leu Ser Lys Leu Asp Lys Ala Ser Leu Gln Ala Gln 230 235 Arg Lys Leu Thr Ile Pro Phe Val Lys Glu Ile Leu Lys Leu 245 <210> 11 <211> 2060 <212> DNA <213> Pasteurella multocida <220> <221> CDS <222> (856)..(1389) <220> <223> dsbB <400> 11 gaattettet taegtatget eccagteaeg tigeeagtie teattigigg tittagigace 60 tgcttcttag tggaaaaatt tggtgtattt ggctatggcg ccaaattgcc acgtaaagta 120 tggggcatct tggcaaagtt tgatcgcaat aatcaacaaa aaatgtcacg acaagatcgt 180 ttgaaacttt ttgtgcaage tttaattggt atttggttgg ttgttggact cgcattecat 240 ctcgccgccg tcggtatcat tggtttaacg gtgattattt tggctacttc attttgtggt 300 gtcaccagcg agcatgctat tggtaaagcc tttcaggaat ccttaccctt cacagcattg 360 ttagtggtgt tetteteggt tgttgeegte ateattgace aacatetgtt tgegeeaatt 420 attcagtttg tgctggctgc cagtgaacat actcagcttg ctcttttcta tatttttaac 480 ggtttgttat ccgccatttc agataatgtg tttgtggcca cagtttatat caatgaaacc 540 aaageggeat tagaggetgg ettaattget caaceacaat atgaattaet ggeagtagea 600 attaatacog gtaccaatot teettetgtt geaaceecaa atggteaage egeattetta 660 tttttattga cctcatcact ggcaccatta attcgtcttt cttatggtag aatggtttat 720 atggcattgc cttataccat cgtattatcc tgtattggtt tattgactgt ggaatatatt 780 ttgcctggcg caaccaatgt gctcattcaa attggtttat taaaaccaat gtaatgacaa 840

Met Leu Ser Phe Phe Lys Thr Leu Ser Thr Lys Arg

gtaaaaggag gaaac atg cta agc ttt ttt aag aca ctc tca aca aaa cga 891

5

1

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					cat His											987
					gct Ala 50											1035
					agt Ser											1083
					gca Ala											1131
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Val Ala Ile Leu Gly Ile Ala Phe Ser Gly Leu Leu Gly Leu Leu Tyr
50 55 60

Pro Ser Ser Met Leu Leu Arg Leu Val Ala Leu Leu Ile Gly Leu Ser 65 70 75 80

Ser Ala Ile Lys Gly Leu Met Ile Ser Ile Thr His Leu Asp Leu Gln 85 90 95

Leu Tyr Pro Ala Pro Trp Lys Gln Cys Ser Ala Val Ala Glu Phe Pro 100 105 110

Glu Thr Leu Pro Leu Asp Gln Trp Phe Pro Ala Leu Phe Leu Pro Ser 115 120 125

Gly Ser Cys Ser Glu Val Thr Trp Gln Phe Leu Gly Phe Ser Met Val 130 135 140

Gln Trp Ile Val Val Ile Phe Ala Leu Tyr Thr Leu Leu Leu Ala Leu 145 150 155 160

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Lys Lys Ala Lys Gln Gln Ala 150

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Glu Arg Phe Leu Phe Leu Ser Arg Val Asn Val Ala Ser Tyr Glu Ser 35 40 45

Ile His Glu Leu Asp Ile Asp Leu Gln Arg His Leu Thr Ala Ile Ser

Thr Ile Gly Ser Asn Ala Pro Tyr Val Gly Leu Leu Gly Thr Val Ile

65 70 75 80 Gly Ile Leu Leu Thr Phe Tyr Glu Leu Gly His Ser Gly Gly Asp Ile Asp Ala Ala Ala Ile Met Val His Leu Ser Leu Ala Leu Lys Ala Thr Ala Val Gly Ile Leu Val Ala Ile Pro Ala Met Val Cys Tyr Asn Gly 120 Leu Gly Arg Lys Val Glu Val Asn Arg Leu Lys Trp Phe Ala Leu Asn 135 140 Glu Lys Lys Ala Lys Gln Gln Ala 150 <210> 15 <211> 6876 <212> DNA <213> Pasteurella multocida <220> <221> CDS <222> (534)..(6863) <220> <223> fhaB1 <400> 15 agatgcgtga tctgatcctt caactcagca aaagttcgat ttattcaaca aagccqccqt 60 cccgtcaagt cagcgtaatg tctgccagtg ttacaccaat taaccaattc tgattagaaa 120 aactcatcga gcatcaaatg aaactgcaat ttattcatat caggattatc aataccatat 180 ttttgaaaaa gccgtttctg taatgaagga gaaaactcac cgaggcagtt ccataggatg 240 gcaagateet ggtateggte tgegatteeg actegteeaa cateaataea acetattaat 300 ttcccctcgt caaaaataag gttatcaagt gagaaatcac catgagtgac gactgaatcc 360 ggtgagaatg gcaaaagctt atgcatttct ttccagactt gttcaacagg ccagccatta 420 egetegteat caaaateact egeateaace aaacegttat teattegtga ttgegeetga 480 gcgagacgaa atacgcgatc gctgttaaaa ggacaattac aaacaggaat cga atq 536 Met 1 caa ccg gcg cag gaa cac tgc cag cgc atc aac aat att gtt aac caa Gln Pro Ala Gln Glu His Cys Gln Arg Ile Asn Asn Ile Val Asn Gln gaa aac ggt tta ttc cat aca ctc ggt aat atg atg tta gaa gca gag 632 Glu Asn Gly Leu Phe His Thr Leu Gly Asn Met Met Leu Glu Ala Glu cgt tct gtt tat aat att ggc gat att tat gcg agt aaa aaa tta aca 680 Arg Ser Val Tyr Asn Ile Gly Asp Ile Tyr Ala Ser Lys Lys Leu Thr 35 40 45

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Thr Val His Thr His Asn Leu Ile Asn Asp Val Arg Leu Ser Gly Asn 50 55 60

Val Ser Tyr Lys Pro Ile Gly Ser Ser Arg Asp Tyr Asp Ile Ser Arg 65 70 75 80

Val Ala Val His Gly Trp His Asn Asn Val Tyr Lys Leu Asn Leu Asn 85 90 95

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100 105 110

Ile Arg Ser Asp Gly Asp Phe Asp Phe Lys Gly Ile Lys Ala Thr Ser 115 120 125

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Phe Asn Gln Asn Ala Leu Ala Ser Val Phe Lys Asn Pro Ala Lys Ile 165 170 175

Thr Met Tyr Tyr Gln Pro Leu Thr Arg Tyr Ile Trp Thr Pro Leu Ser 180 185 190

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Leu Phe Gly Ser Thr Thr Ile Leu Lys Ser Ser Phe Tyr Ser Thr Glu 210 215 220

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- His Gly Lys Leu His Val Leu Gly Tyr Ala Asp Ile Gly Gly Val Asp 1090 1095 1100
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- Lys Gln Lys Asp Gln Tyr Asp His Glu Ser Glu Arg Thr Thr Phe Lys 1205 1210 1215
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- Thr Pro Thr Gly Asn Val Gly Phe Gly Tyr Thr Asn Glu Thr Glu Ser 1460 1465 1470
- Lys Arg Thr Val Asn Gln Gln Ala Gly Ile Lys Ala Asn Lys Ile Thr 1475 1480 1485
- Gly Gln Thr His Asp Leu Asn Leu Glu Gly Gly Tyr Leu Val Ser Asn 1490 1495 1500
- Asp Lys Asp Asn Gln Leu Lys Val Thr Gly Asp Val Thr Thr Lys Ala 1505 1510 1515 1520
- Leu His Asp Gln His Asp Lys Asp Gly Gly Thr Phe Gly Leu Ser Val 1525 1530 1535
- Gly Ile Ser Glu Arg Gly Thr Thr Ala Phe Asn Val Arg Gly Gly Arg 1540 1545 1550
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- Thr Lys Ala Lys Ala Val Thr Arg Asp Asp Thr Tyr Ala Ser Thr Gln 1585 1590 1595 1600
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- Ser Asp Glu His Leu Tyr Ala Glu Ile Asn Glu Pro Thr Tyr Ser Arg 1730 1735 1740
- Val Gly Asp Lys Asn Ala Asp Met Arg Arg His Asn Ala Ala Gly Thr 1745 1750 1755 1760
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- Gly Ser Glu His Ile Tyr Thr Asp Ile Ser Asp Val Gly Thr Gln Thr 1795 1800 1805
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- Ala Val Asn Leu Ile Gly Gln Asn Gly Leu Gly Ser Ile Tyr His Ser 1825 1830 1835 1840
- Pro Asp Ser Ala Tyr Lys Thr Trp Gl \dot{n} Leu Leu Asp Gln Phe Ala Asn 1845 1850 1855
- Lys Gly Gly Asp Ala Val Phe Leu Arg Pro Ala Thr Glu Met Lys Cys 1860 1865 1870
- Ala Gly Ala Pro Leu Lys Tyr Thr Phe Ile Val Arg Asp Tyr Leu Leu 1875 1880 1885
- Arg Arg His Thr Leu Asp Lys Ser Arg Leu Phe Tyr Asn Ala His Asn 1890 1895 1900
- Lys Thr Leu Phe Ser Val Pro Ile Val Asp Ala Lys Val Lys Met Leu 1905 1910 1915 1920
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Val Glu Val Pro Tyr Asp Phe Ile Asn Val Val Pro Pro Met Arg Ala 1955 1960 1965

Pro Asp Ala Val Arg Gln Ser Ala Leu Ala Trp Gln Glu Gly Lys Trp 1970 1975 1980

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Arg Tyr Ala Asn Val Phe Ala Val Gly Asp Val Ala Gly Val Pro Lys 2005 2010 2015

Gly Lys Thr Ala Ala Ser Val Lys Trp Gln Val Pro Val Ala Val Ala 2020 2025 2030

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		aat Asn								1976
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Asp Lys Gly Ala Gly Val Lys His Asp Gly Ile Ile Leu Ser Glu Asn Asp Ile Gln Ile Glu Met Asn Glu Gly Asp Leu Glu Leu Gly Asn Thr Ile Gln Gln Thr Val Val Lys Lys Asp Arg Asn Ile Arg Ala Lys Lys Lys Ile Glu Val Lys Asn Ala Asn Arg Val Phe Val Gly Ser Gln Thr 405 410 Lys Ser Asp Glu Ile Ser Leu Glu Ala Lys Gln Val Lys Ile Arg Lys 425 Asn Ala Glu Ile Arg Ser Thr Thr Gln Ala Lys Ile Val Ala Lys Gly 440 Ala Leu Ser Ile Glu Gln Asn Ala Lys Leu Val Ala Lys Lys Ile Asp Val Ala Thr Glu Thr Leu Thr Asn Ala Gly Arg Ile Tyr Gly Arg Glu 470 Val Lys Leu Asp Thr Asn Asn Leu Ile Asn Asp Lys Glu Ile Tyr Ala 485 Glu Arg Lys Leu Ser Ile Leu Thr Lys Gly Lys Asp Leu Glu Ile Ile Gln Asp Arg Tyr Leu Ser Pro Leu Met Arg Val Lys Ser Ser Val Arg Phe Leu Gly Ser Pro Phe Phe Ser Ile Ser Pro Ser Met Leu Ala Ser Leu Ser Ala Gln Phe Lys Pro Gly Phe Val Asn Lys Gly Leu Ile Glu 550 555 Ser Ala Gly Ser Ala Glu Leu Thr Phe Lys Glu Lys Thr Ser Phe Leu Thr Glu Gly Asn Asn Phe Ile Arg Ala Lys Asp Ala Leu 585 <210> 19 <211> 3247 <212> DNA <213> Pasteurella multocida <220> <221> CDS <222> (1)..(1446) <220> <223> fhaC <400> 19

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							gat Asp 110										992
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- Val Lys Lys Asn Gly Phe Ala Ser Phe Pro Val Val Asp Asp Glu Lys
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- Thr Val Lys Arg Asn Ala Ser Arg Asp Glu Ile Phe Gly Leu Met His
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- Thr His Arg Val Glu Lys Val Leu Val Val Ser Asp Asp Phe Lys Leu 180 185 190
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Pro Arg Asn Ala Thr Pro Gln Glu Glu Lys Leu Gln Val Glu Ile Asp 100 105 110

Glu Leu Phe Tyr Gln Phe Pro Met Leu Glu Asp Leu Met Val Asp Met 115 120 125

Met Asp Ala Val Gly His Gly Phe Ser Ala Leu Glu Ile Glu Trp Lys 130 135 140

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Ser Trp Phe Lys Leu Asp Lys Asp Asp Asn Leu Leu Lys Thr Pro 165 170 175

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					cca Pro									6759
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			ttc act tac ca Phe Thr Tyr Gl		7239					
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			agt gct gtt cg Ser Ala Val Arg 885		431					
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Glu Lys Lys Ile Gly Glu Thr Val Lys Thr Ala Ser Gln Leu Lys Arg 50 55 60

Gln Gln Val Gln Asp Ser Arg Asp Leu Val Arg Tyr Glu Thr Gly Val 65 70 75 80

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Glu Lys Ala Asp Pro Tyr Thr Ile Thr Lys Glu Ser Thr Leu Val Lys 245 250 255

Phe Ser Phe Ser Pro Thr Glu Asn His Arg Phe Thr Val Ala Ser Asp 260 265 270

Thr Tyr Leu Gln His Ser Arg Gly His Asp Leu Ser Tyr Asn Leu Val 275 280 285

Ala Thr Thr His Ile Gln Leu Asp Glu Lys Glu Ser Arg His Ala Asn 290 295 300

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Tyr Ala Lys Phe Gly Glu Ala Tyr Lys Lys Trp Lys Glu Tyr Leu Pro 645 650 Lys Asn Ala Glu Glu Asn Ile Ala Tyr Ile Ala Gln Asp Lys Thr Phe Lys Lys His Ser Tyr Ser Leu Gly Ala Thr Phe Asp Pro Leu Asn Phe Leu Arg Val Gln Val Lys Tyr Ser Lys Gly Phe Arg Ala Pro Thr Ser Asp Glu Leu Tyr Phe Thr Phe Lys His Pro Asp Phe Thr Ile Leu Pro Asn Pro Val Leu Lys Pro Glu Glu Ala Lys Asn Gln Glu Ile Ala Leu Thr Val His Asp Asn Trp Gly Phe Val Ser Thr Ser Val Phe Gln Thr Lys Tyr Arg His Phe Ile Asp Leu Ala Tyr Leu Gly Ser Arg Asn Leu Ser Asn Ser Val Gly Gly Gln Ala Gln Ala Arg Asp Phe Gln Val Tyr Gln Asn Val Asn Val Asp Asn Ala Lys Val Lys Gly Leu Glu Ile Asn Ala Arg Leu Asn Leu Gly Tyr Phe Trp His Val Leu Asp Gly Phe Asn Thr Ser Tyr Lys Phe Thr Tyr Gln Arg Gly Arg Leu Asp Gly Asp Arg 825 Pro Met Asn Ala Ile Gln Pro Lys Ala Ser Val Phe Gly Leu Gly Tyr Asp His Lys Glu Asn Lys Phe Gly Ala Asp Leu Tyr Ile Thr Arg Val Ser Glu Lys Lys Ala Lys Asp Thr Tyr Asn Met Phe Tyr Lys Glu Gln Gly Tyr Lys Asp Ser Ala Val Arg Trp Arg Ser Asp Asp Tyr Thr Leu Val Asp Ala Val Gly Tyr Ile Lys Pro Ile Lys Asn Leu Thr Leu Gln 905 Phe Gly Val Tyr Asn Leu Thr Asp Arg Lys Tyr Leu Thr Trp Glu Ser Ala Arg Ser Ile Lys Pro Phe Gly Thr Ser Asn Leu Ile Asn Gln Lys 935 Thr Gly Ala Gly Ile Asn Arg Phe Tyr Ser Pro Gly Arg Asn Phe Lys 955 Leu Ser Ala Glu Ile Thr Phe 965

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Tyr Phe Leu Trp Phe Ile Leu Phe Ile Leu Ser Ile Tyr Leu Phe Ile
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Thr Ile Gln Glu Arg Arg Gly Tyr Cys Phe Asp Lys Arg Ala Tyr Ile
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cca gct tg Pro Ala Cy 90	t tgt aca t s Cys Thr I	ta acc ac Leu Thr Th 95	c ttt r Phe	att gat Ile Asp 100	Glu Gly	ggc gat Gly Asp	ggc Gly 105	1420
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Tyr Cys Phe Asp Lys Arg Ala Tyr Ile His Glu Leu Tyr Thr Glu Gln
35 40 45

Glu Leu Ile Asp Arg Gly Ile Glu Tyr Val Val Ser Thr Met Pro Ser 50 60

Gly Val Ile Lys Pro Asp Gly Thr Ile Lys Glu Val Lys Arg Tyr Thr 65 70 75 80

Ser Val Glu Glu Phe Lys Gln Met Asn Pro Ala Cys Cys Thr Leu Thr

Thr Phe Ile Asp Glu Gly Gly Asp Gly Tyr Pro Asp Asp Gly Tyr
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Val Asn Phe Ile Ala Val Asn Glu Lys Glu Phe Glu Ser Phe Pro Val
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Gln Ile Ser Tyr Ile Ser Leu Ala Thr Tyr Ala Arg Leu Lys Ala Ala
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gta ttc tca Val Phe Ser 170											879
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Glu Lys Glu Phe Glu Ser Phe Pro Val Gln Ile Ser Tyr Ile Ser Leu
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Ala Thr Tyr Ala Arg Leu Lys Ala Ala Glu Tyr Leu Pro Asp Asn Leu 85 90 95

Asn Lys Ile Ile Tyr Leu Asp Val Asp Val Leu Val Phe Asn Ser Leu 100 105 110

Glu Met Leu Trp Asn Val Asp Val Asn Asn Phe Leu Thr Ala Ala Cys 115 120 125

Tyr Asp Ser Phe Ile Glu Asn Glu Lys Ser Glu His Lys Lys Ser Ile 130 _ 135 140

Ser Met Ser Asp Lys Glu Tyr Tyr Phe Asn Ala Gly Val Met Leu Phe 145 155 Asn Leu Asp Glu Trp Arg Lys Met Asp Val Phe Ser Arg Ala Leu Asp Leu Leu Ala Met Tyr Pro Asn Gln Met Ile Tyr Gln Asp Gln Asp Ile Leu Asn Ile Leu Phe Arg Asn Lys Val Cys Tyr Leu Asp Cys Arg Phe Asn Phe Met Pro Asn Gln Leu Glu Arg Ile Xaa Gln Tyr His Lys Gly 215 Lys Xaa Ser Asn Leu His Ser Leu Glu Lys Thr Thr Met Pro Val Val 225 235 230 Ile Ser His Tyr Cys Gly Pro Glu Lys Ala Trp His Ala Asp Cys Lys 250 His Phe Asn Val Tyr Phe Tyr Gln Lys Ile Leu Ala Xaa Xaa Ser Arg 260 265 Gly Xaa Asp Lys Glu Arg Val Leu Ser Ile Lys Thr Tyr Leu Lys Ala Leu Ile Arg Arg Ile Arg Tyr Lys Phe Lys Tyr Gln Val Tyr 295 <210> 37 <211> 2029 <212> DNA <213> Pasteurella multocida <220> <221> CDS <222> (2)..(499) <220> <223> mglB <220> <221> misc_feature <222> 33 <223> Xaa = any or unknown amino acid <220> <221> misc_feature <222> 99 <223> Xaa = any or unknown amino acid <220> <221> misc_feature <222> 101 <223> Xaa = any or unknown amino acid <220> <221> misc_feature <222> 928 <223> n = A or T or G or C

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Gly His Pro Asp Ala Glu Ala Arg Thr Lys Phe Val Ile Lys Glu Leu
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Ser Lys Ala Asn Gln Ile Glu Val Ile Ile Ala Asn Asn Asp Gly Met
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Ile Phe Xaa Val Xaa Ala Leu Pro Glu Val Leu Gln Leu Ile Lys Lys
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Gly Glu Ile Ala Gly Thr Val Leu Asn Asp Gly Val Asn Gln Gly Lys
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Ser Lys Ala Asn Gln Ile Glu Val Ile Ile Ala Asn Asn Asp Gly Met
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Ile Phe Xaa Val Xaa Ala Leu Pro Glu Val Leu Gln Leu Ile Lys Lys
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<212> PRT

<213> Pasteurella multocida

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Gly Arg Thr Pro Lys Ser Ile Ala Ala Ile Arg Pro Met Lys Asp Gly 65 70 75 80

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Lys Gln Val His Ser Ser Asn Phe Met Arg Pro Ser Pro Arg Val Leu 100 105 110

Val Cys Val Pro Ala Gly Ala Thr Gln Val Glu Arg Arg Ala Ile Lys 115 120 125

Glu Ser Ala Ile Gly Ala Gly Ala Arg Glu Val Tyr Leu Ile Glu Glu 130 135 140

Pro Met Ala Ala Ile Gly Ala Lys Leu Pro Val Ser Thr Ala Thr 145 150 155 160

Gly Ser Met Val Ile Asp Ile Gly Gly Gly Thr Thr Glu Val Ala Val 165 170 175

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Ser Ile Ile Gly Glu Pro Thr Ala Glu Arg Ile Lys Gln Glu Ile Gly
210 215 220

Ser Ala Phe Ile Gln Glu Gly Asp Glu Val Arg Glu Ile Glu Val His 225 230 235 240

Gly His Asn Leu Ala Glu Gly Ala Pro Arg Ser Phe Lys Leu Thr Ser

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atc gta ggt aac aaa gaa ggc tta gtg cat att tct caa atc gcg gaa 1296 Ile Val Gly Asn Lys Glu Gly Leu Val His Ile Ser Gln Ile Ala Glu 420 425 430
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Asp Pro Lys Lys Ile Lys Asp Val Ile Gly Lys Gly Gly Ala Thr Ile 340 345 Arg Ala Leu Thr Glu Glu Thr Gly Thr Ser Ile Asp Ile Asp Asp Asp 360 Gly Thr Val Lys Ile Ala Ala Val Asp Gly Asn Ser Ala Lys Glu Val Met Ala Arg Ile Glu Asp Ile Thr Ala Glu Val Glu Ala Gly Ala Val Tyr Lys Gly Lys Val Thr Arg Leu Ala Asp Phe Gly Ala Phe Val Ser Ile Val Gly Asn Lys Glu Gly Leu Val His Ile Ser Gln Ile Ala Glu Glu Arg Val Glu Lys Val Ser Asp Tyr Leu Ala Val Gly Gln Glu Val Thr Val Lys Val Val Glu Ile Asp Arg Gln Gly Arg Ile Arg Leu Thr 455 Met Lys Glu Val Ala Pro Lys Gln Glu His Val Asp Ser Val Val Ala Asp Val Ala Ala Glu Glu Asn Ala 485 <210> 45 <211> 633 <212> DNA <213> Pasteurella multocida <220> <221> CDS <222> (2)..(631) <220> <223> purF <400> 45 c gat ggg gtt tct gtt tat gct gcc cgt gtt cat atg gga caa cgt tta 49 Asp Gly Val Ser Val Tyr Ala Ala Arg Val His Met Gly Gln Arg Leu ggt gaa aaa att gca cgg gaa tgg gcg gat gtg gat gat att gat gtg 97 Gly Glu Lys Ile Ala Arg Glu Trp Ala Asp Val Asp Asp Ile Asp Val gtc att cct gtg cct gaa acc tct aac gat att gct tta cgt att gcg 145 Val Ile Pro Val Pro Glu Thr Ser Asn Asp Ile Ala Leu Arg Ile Ala 40 cgc gtg tta aat aaa ccg tat cgt caa ggt ttt gtg aaa aat cgc tat 193 Arg Val Leu Asn Lys Pro Tyr Arg Gln Gly Phe Val Lys Asn Arg Tyr gta gga cgt acg ttt att atg ccg ggg cag gca ttg cga gtc agt tct 241

75

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										gtg Val						433
										cgt Arg 155						481
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Arg Tyr Asp Ile Ser Asn Leu Tyr Ile Arg Asp Leu Arg Lys Glu Asp
50 55 60

Phe Glu Glu Trp Ile Arg Ile Arg Leu Thr Glu Val Ser Asp Ala Ser 65 70 75 80

Val Arg Arg Glu Leu Val Thr Ile Ser Ser Val Leu Thr Thr Ala Ile 85 90 95

Asn Lys Trp Gly Tyr Ile Ser Arg His Pro Met Thr Gly Ile Glu Lys 100 105 110

Pro Lys Asn Ser Ala Glu Arg Lys Glu Arg Tyr Ser Glu Gln Asp Ile 115 120 125

Lys Thr Ile Leu Glu Thr Ala Arg Tyr Cys Glu Asp Lys Leu Pro Ile 130 135 140

Thr Leu Lys Gln Arg Val Ala Ile Ala Met Leu Phe Ala Ile Glu Thr 145 150 155 160

Ala Met Arg Ala Gly Glu Ile Ala Ser Ile Lys Trp Asp Asn Val Phe
165 .170 .175

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Arg Asp Val Pro Leu Ser Gln Arg Ala Val Ala Leu Ile Leu Lys Met 195 200 205

Lys Glu Val Glu Asn Gly Asp Leu Val Phe Gln Thr Thr Pro Glu Ser 210 215 220

Leu Ser Thr Thr Phe Arg Val Leu Lys Lys Glu Cys Gly Leu Glu His 225 230 235 240

Leu His Phe His Asp Thr Arg Arg Glu Ala Leu Thr Arg Leu Ser Lys 245 250 255

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aat caa gca att cgc aca att caa agt cta tca acc gca gtc atc ggt 97 Asn Gln Ala Ile Arg Thr Ile Gln Ser Leu Ser Thr Ala Val Ile Gly 20 25 30

att gtc tgt act gca aat gac gca gac aat gaa aca ttc cca ctc aat 149
Ile Val Cys Thr Ala Asn Asp Ala Asp Asn Glu Thr Phe Pro Leu Asn
35 40 45

gaa ccc gtt ctc atc aca aac gtg gca gcg gca att ggc aag gct gga 193 Glu Pro Val Leu Ile Thr Asn Val Ala Ala Ala Ile Gly Lys Ala Gly

aaa caa ggc acg ctt tca cgt gcg ctt gac ggg att tct gat gta gtc 241 Lys Gln Gly Thr Leu Ser Arg Ala Leu Asp Gly Ile Ser Asp Val Val 65 70 75 80

aat tgc aaa gtg att gtt gtg cga gtg caa gaa agt gcg caa gaa gac 289 Asn Cys Lys Val Ile Val Val Arg Val Gln Glu Ser Ala Gln Glu Asp 85 90 95

gaa gaa aca aaa gca agt gaa atg aac acg gca att att ggc aca atc 337 Glu Glu Thr Lys Ala Ser Glu Met Asn Thr Ala Ile Ile Gly Thr Ile 100 105 110

aca gaa gaa ggg cag tac aca ggc ttg aag gcg tta ttg att gcg aaa 385 Thr Glu Glu Gly Gln Tyr Thr Gly Leu Lys Ala Leu Leu Ile Ala Lys 115 120 125

aac aaa ttc ggt atc aaa cca cgt att tta tgt gtg cca aaa ttc gac 433 Asn Lys Phe Gly Ile Lys Pro Arg Ile Leu Cys Val Pro Lys Phe Asp 130 140

aca aaa gaa gtc gcc aca gag ctt gca agt atc gcc gcc aaa ctc aac 481 Thr Lys Glu Val Ala Thr Glu Leu Ala Ser Ile Ala Ala Lys Leu Asn 150 155 160

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gtg caa tat aaa cgc aac ttc tca caa cgt gaa gtc atg ctg atc atg 577 Val Gln Tyr Lys Arg Asn Phe Ser Gln Arg Glu Val Met Leu Ile Met 180 185

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gca ggg gcg ttt gat tgg gca gtg gat aaa gat att tct gtc acg cta 961 Ala Gly Ala Phe Asp Trp Ala Val Asp Lys Asp Ile Ser Val Thr Leu 305 310 315 320	L
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Ile	Val	Cys 35	Thr	Ala	Asn	Asp	Ala 40	Asp	Asn	Glu	Thr	Phe 45	Pro	Leu	Asn
Glu	Pro 50	Val	Leu	Ile	Thr	Asn 55	Val	Ala	Ala	₽.1a	Ile 60	Gly	Lys	Ala	Gly
Lys 65	Gln	Gly	Thr	Leu	Ser 70	Arg	Ala	Leu	Asp	Gly 75	Ile	Ser	Asp	Val	Val 80
Asn	Cys	Lys	Val	Ile 85	Val	Val	Arg	Val	Gln 90	Glu	Ser	Ala	Gln	Glu 95	Asp
Glu	Glu	Thr	Lys 100	Ala	Ser	Glu	Met	Asn 105	Thr	Ala	Ile	Ile	Gly 110	Thr	Ile
Thr	Glu	Glu 115	Gly	Gln	Tyr	Thr	Gly 120	Leu	Lys	Ala	Leu	Leu 125	Ile	Ala	Lys
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Thr 145	Lys	Glu	Val	Ala	Thr 150	Glu	Leu	Ala	Ser	Ile 155	Ala	Ala	Lys	Leu	Asn 160
Ala	Phe	Ala	Tyr	Ile 165	Ser	Cys	Gln	Gly	Cys 170	Lys	Thr	Lys	Glu	Gln 175	Ala
Val	Gln	Tyr	Lys 180	Arg	Asn	Phe	Ser	Gln 185	Arg	Glu	Val	Met	Leu 190	Ile	Met
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Tyr	Ala 210	Val	Thr	Arg	Ala	Ala 215	Ala	Met	Arg	Ala	Tyr 220	Leu	Asp	Lys	Glu
Gln 225	Gly	Trp	His	Thr	Ser 230	Ile	Ser	Asn	Lys	Gly 235	Ile	Asn	Gly	Val	Ser 240
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Lys	Phe 290	Glu	Val	Tyr	Thr	Arg 295	Thr	Ala	Gln	Ile	Leu 300	Lys	Asp	Thr	Ile
Ala 305	Gly	Ala	Phe	Asp	Trp 310	Ala	Val	Asp	Lys	Asp 315	Ile	Ser	Val	Thr	Leu 320
Val	Lys	Asp	Ile	Ile 325	Glu	Ala	Ile	Asn	Ala 330	Lys	Trp	Arg	Asp	Tyr 335	Thr

Thr Lys Gly Tyr Leu Ile Gly Gly Lys Ala Trp Leu Asn Lys Glu Leu

Asn Ser Ala Thr Asn Leu Lys Asp Ala Lys Leu Leu Ile Ser Tyr Asp Tyr His Pro Val Pro Pro Leu Glu Gln Leu Gly Phe Asn Gln Tyr Ile Ser Asp Glu Tyr Leu Val Asp Phe Ser Asn Arg Leu Ala Ser <210> 51 <211> 353 <212> DNA <213> Pasteurella multocida <220> <221> CDS <222> (1)..(351) <220> <223> unknown C1 <400> 51 atg aca tta ttt gat gaa tgt aaa tta gct ctt aga gac gat ttt aat Met Thr Leu Phe Asp Glu Cys Lys Leu Ala Leu Arg Asp Asp Phe Asn cta att tgt gat gaa gag aag gat tgt gta atg gat aag ttt tat ttc Leu Ile Cys Asp Glu Glu Lys Asp Cys Val Met Asp Lys Phe Tyr Phe 25 tat ttc ttg gaa aag aaa gag gaa ttt aat ttt caa gat tat tca ttt 144 Tyr Phe Leu Glu Lys Lys Glu Glu Phe Asn Phe Gln Asp Tyr Ser Phe 40 gaa gaa atg tat ata ttt tca aaa atg gaa cct gtg tat gtt tta tgt Glu Glu Met Tyr Ile Phe Ser Lys Met Glu Pro Val Tyr Val Leu Cys gat age tet aat ata eet tig tit agg agt aat igg gaa tig att ate Asp Ser Ser Asn Ile Pro Leu Phe Arg Ser Asn Trp Glu Leu Ile Ile 65 aat aat ata tat gat gtt gtc tgt tta tct aca aaa gta ttt ttt cta 288 Asn Asn Ile Tyr Asp Val Val Cys Leu Ser Thr Lys Val Phe Phe Leu gat gat gaa aag tta atg atg gaa tta ttt cct gaa gat aaa gta aga Asp Asp Glu Lys Leu Met Met Glu Leu Phe Pro Glu Asp Lys Val Arg 100 105 gtc atc tat aaa aga ta 353 Val Ile Tyr Lys Arg 115 <210> 52 <211> 117 <212> PRT <213> Pasteurella multocida

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tat gaa cat gtg tat tot ttt ggt agt act ggc gag gga cat ttt atc Tyr Glu His Val Tyr Ser Phe Gly Ser Thr Gly Glu Gly His Phe Ile 105 100 tgt ttt gat tat cgt gat gat cca aaa ggt gat gaa ccc aaa atc tgt 384 Cys Phe Asp Tyr Arg Asp Asp Pro Lys Gly Asp Glu Pro Lys Ile Cys atc gtg att cac gat gaa tat gat gaa aaa aca ggg aaa atg cga ctg 432 Ile Val Ile His Asp Glu Tyr Asp Glu Lys Thr Gly Lys Met Arg Leu 130 135 ttt cct ata gca gag aat ttt gaa gcg ttt tta gat agt ttg aaa tca Phe Pro Ile Ala Glu Asn Phe Glu Ala Phe Leu Asp Ser Leu Lys Ser 150 ttt gat gaa atg ata gag aag tat tcg ta 509 Phe Asp Glu Met Ile Glu Lys Tyr Ser 165

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Asp Lys Glu Ile Ile Leu Glu Phe Glu Asn Glu Phe Asn Ile Lys Leu 20 25 30

Pro Ser Leu Tyr Ile Asp Leu Ile Thr Ala His Asn Ala Pro Lys Ser 35 40 45

Glu Glu Asn Cys Phe Glu Tyr Tyr Asn Glu Arg Asn Glu Pro Thr Phe
50 60

Ser Ser Phe Gly Phe Glu Gly Phe Glu Thr Glu Arg Ser Ser Ala Ser 65 70 75 80

Leu Glu Asn Ile Tyr Ala Gln Tyr Ile Tyr Asp Asp Pro Ile Tyr Gly
85 90 95

Tyr Glu His Val Tyr Ser Phe Gly Ser Thr Gly Glu Gly His Phe Ile 100 105 110

Cys Phe Asp Tyr Arg Asp Asp Pro Lys Gly Asp Glu Pro Lys Ile Cys 115 120 125

Ile Val Ile His Asp Glu Tyr Asp Glu Lys Thr Gly Lys Met Arg Leu 130 135 140

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Gly Lys Asn Glu Ser Asn Lys Asp Ile Leu Lys Leu Val Glu Ile Val
tct tca gat ttt gaa gtg gat gaa cta agt cat aaa gat gaa cac gag
                                                                144
Ser Ser Asp Phe Glu Val Asp Glu Leu Ser His Lys Asp Glu His Glu
ata tat tat ttg ttt tat aag agg ggt gtt gaa ttt tgt ttt aaa aga
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Ile Tyr Tyr Leu Phe Tyr Lys Arg Gly Val Glu Phe Cys Phe Lys Arg
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ata gat gaa gag tat gtc tta tat tcg gtt ttc ttt ttc ttg gta gag
Ile Asp Glu Glu Tyr Val Leu Tyr Ser Val Phe Phe Leu Val Glu
                    70
65
gtt gat aat tat ttt tca tgc cca ttt att cat gaa tta ata tgt gat
Val Asp Asn Tyr Phe Ser Cys Pro Phe Ile His Glu Leu Ile Cys Asp
ctt aaa cac gga ttc tca ata gag gat att ata agg ttt tta ggg gag
                                                                336
Leu Lys His Gly Phe Ser Ile Glu Asp Ile Ile Arg Phe Leu Gly Glu
cca aat ttt aaa ggt agt ggc tgg gta aga tat tct tat aat gga aga
                                                                384
Pro Asn Phe Lys Gly Ser Gly Trp Val Arg Tyr Ser Tyr Asn Gly Arg
aat att cat ttc gaa ttt aat gaa tct aat gaa tta tcc cag att agc
                                                                432
Asn Ile His Phe Glu Phe Asn Glu Ser Asn Glu Leu Ser Gln Ile Ser
   130
                       135
att ttt att ta
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Ile Phe Ile
145
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Gly Lys Asn Glu Ser Asn Lys Asp Ile Leu Lys Leu Val Glu Ile Val
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Ile Tyr Tyr Leu Phe Tyr Lys Arg Gly Val Glu Phe Cys Phe Lys Arg 50 60

Ile Asp Glu Glu Tyr Val Leu Tyr Ser Val Phe Phe Leu Val Glu 65 70 75 80

Val Asp Asn Tyr Phe Ser Cys Pro Phe Ile His Glu Leu Ile Cys Asp 85 90 95

Leu Lys His Gly Phe Ser Ile Glu Asp Ile Ile Arg Phe Leu Gly Glu 100 105 110

Pro Asn Phe Lys Gly Ser Gly Trp Val Arg Tyr Ser Tyr Asn Gly Arg 115 120 125

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Ile	Gln	Gly 115	Ile	Ala	Lys	Leu	Tyr 120	Leu	Arg	Ser	Glu	Asn 125	Ala	Asn	Ala
Ser	Ser 130	Asp	Ala	Pro	Ile	Thr 135	Ile	Asp	Lys	Pro	Phe 140	His	Tyr	Ser	Cys
Glu 145	Glu	Leu	Asp	Leu	Pro 150	Thr	Ala	Asn	Glu	Tyr 155	Ala	Arg	Arg	Lys	Pro 160
Ile	Val	Cys	Glu	Val 165	Gln	Gly	Gly	Val	Asn 170	Arg	ГЛЗ	Phe	Trp	Leu 175	Pro
Val	Ser	Glu	Ser 180	Leu	Val	Ser	Glu	Asp 185	Lys	Leu	Lys	Lys	Asp 190	Arg	Va]
Arg	Leu	Glu 195	Ser	Asp	Thr	Ser	Tyr 200	Ala	Ile	Lys	Glu	Lys 205	Gly	Ile	Va]
Ile	Pro 210	Val	Glu	Leu	Met	Leu 215	Val	Ser	Asp	Tyr	Ser 220	Gly	Ser	Met	Asr
Ser 225	His	Leu	Gln	Asp	Lys 230	Asn	Gly	Arg	Ser	Leu 235	Gly	Lys	Ala	Lys	11e
Thr	Ile	Leu	Arg	Glu 245	Val	Val	Ser	Glu	Ile 250	Ser	Lys	Ile	Leu	Leu 255	Pro
Glu	Asp	Val	Ser 260	Glu	Gly	Val	Ser	Pro 265	Phe	Asn	Arg	Île	Gly 270	Phe	Thr
Thr	Phe	Ser 275	Gly	Gly	Val	Arg	Gln 280	Arg	Asp	Val	Thr	Glu 285	Gly	Cys	Val
Leu	Pro 290		Glu	Gly		Ile 295	Ser	Gln	Thr		Arg 300	-	Leu	Thr	Ile
Arg 305	Tyr	Trp	Ile	Thr	Gly 310	Asn	Asn	Thr	Pro	Trp 315	Lys	Phe	Asn	Ala	Gly 320
Arg	Trp	Glu	Arg	Ser 325	Thr	Val	Ser	Phe	Gln 330	Glu	His	Tyr	Lys	Gly 335	Туг
Tyr	Asp	Lys	Phe 340	His	Ser	Ser	Thr	Cys 345	Arg	Gly	Ser	Gly	Ser 350	Ser	Arg
Thr	Cys	Gln 355	Ile	Asp	Ala	Asn	Pro 360	Lys	Lys	Ile	Met	Asp 365	Tyr	Ala	Leu
Lys	Ile 370	Asn	Asp	Trp	Thr	Thr 375	Ile	Arg	Glu	Leu	Phe 380	Asn	Thr	Tyr	Ile

Asp Val Ser Gly Thr Ile Asp Gln Ile Ser Gln Phe Asp Gly Ser Asn

385 390 395 400

Arg Arg Tyr Asp Met Val Phe Thr Asp Glu Glu Arg Cys Leu Gly Gly 405 410 415

Asn Ile Gly Arg Arg Thr Thr Arg Ala Trp Phe Asp Gln Lys Asn Lys 420 425 430

Asp Ile Thr Arg Glu Leu Asn Ile Val Arg Pro Ser Gly Trp Thr Ser 435 440 445

Ala Ser Ser Gly Leu Leu Val Gly Ala Asn Ile Met Met Asp Glu Asn 450 450 460

Lys Asn Pro Asp Ala Gln Pro Ser Lys Leu Gly Thr Asn Ile Gln Arg
465 470 475 480

Val Ile Leu Val Leu Ser Asp Gly Glu Asp Asn Trp Pro Thr Tyr Ser 485 490 495

Thr Leu Thr Thr Leu Leu Asn Asn Gly Met Cys Asp Lys Ile Arg Glu 500 505 510

Gln Leu Gly Lys Leu Gln Asp Pro Asn Leu Arg Glu Leu Pro Gly Arg 515 520 525

Ile Ala Phe Val Ala Phe Gly Tyr Ser Pro Pro Ala Asn Gln Val Ala 530 535 540

Ala Trp Lys Lys Cys Val Gly Asp Gln Tyr Tyr Thr Ala Tyr Ser Lys 545 550 555

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Tyr Leu Asn Gly Val Lys Asn Ser Arg Leu Ile His Asn Leu Val Phe gct tta gtt aaa aat ggt gat ctt gat tat gca aaa gat atc att gtt 1387 Ala Leu Val Lys Asn' Gly Asp Leu Asp Tyr Ala Lys Asp Ile Ile Val aaa gag cgt tta aat act tca cca gat gat tta att aat gca ttg aaa 1435 Lys Glu Arg Leu Asn Thr Ser Pro Asp Asp Leu Ile Asn Ala Leu Lys 235 240 aaa act aca cat gta tca aaa ggt gta act cgg taacactaag gatttgatat 1488 Lys Thr Thr His Val Ser Lys Gly Val Thr Arg 255 gaaaaagttt ctatcaaata taaaaggaac ctcgtcaatt gaatttgctt tqacqatagc 1548 gttctattta tttgttgtga tgtttatttt tgaattttgt cgattagcgg ttgcgacagc 1608 ttattgggat ttagctataa cggaaagtgt cagaattgcg aagaatgaac aagcaatttc 1668 tggaaattat gaagaagcat ttaggaaagc tcttacaaag caaaaaaaat tccatgatga 1728 atcgacaatt ggatatttgg cgttgttaga agataataaa tttgatgtaa aagtccaata 1788 tgtggattgt gataaagaaa cggaatgtat taaaaatctt ctgcttaata aatttcgcca 1848 accacaaaaa aatcataaag gagagttaat ctctcctacg gggagtcgcg cqactttaqc 1908 acaatattot ttaacttata aatataagtt tatggtgccg ttagtattta ttoctgagto 1968 ttggtctcaa gtagtgctga accgtgaatt tgttgttgta caggaatttg aqcgttctca 2028 atttatgtta ggagcaaaac caagttetet tgggacgaat ccatagaaaa tttactcatt 2088 atttcgagct atatatgaaa gagtcaggtt taqttaaatt caaqcatttt tqqaaaaata 2148 aaaagggcgc agtgacgata gagttccttt ttatgtcaat gtttctgatt gtgctatttg 2208 cattletett egattlagta atgttaegtt etacattagg caagttagat aatgeeteat 2268 atacattagt tagtattete egtgaaegta caeagttgta egatagagtt geacaaatta 2328 atattgatga tcataagcaa tttgaaaagc ttgctaagaa actgatttat ggtgatcaga 2388 atagtaataa aaggatcgat gttgttttag aatattgggc acaagacggt tctggacgga 2448 ggattccaaa tatcattggc gattgtacgc cttacaaaaa actttctgat ttatcctatt 2508 tatctcctcg ctcagaactc aataatgaaa gaaaaatacc gctttatcaa attactcttt 2568 gtgttgaaac tcagggcttg tttgaaacaa tattactgga taagtctgag cqttcaacqq 2628 ggctgattag atcatcgtca atgtcagtat cacgataaat tatcgttagg gaactttatg 2688 aaaaaacttt atttaattcg ttcttgctat gattcagtca gaaaatttta tgagaatgag 2748 ctaggtgttt atacagtaat gactgcatta ctagcatttc cattattagt tttgattqqa 2808 tttacggttg atggaactgg ggttgtgctt gataaagcac gtttagctca aggaatggat 2868 caagetgett tagetttggt tgetgaaaac aatgaetace gagaaaataa aaaacatggt 2928

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<213> Pasteurella multocida

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Ser Met Ser Ser Glu Thr Ile Thr Ala Lys Glu Thr Leu Tyr Glu Ser 40 45

Thr Gln Asn Tyr Ser Ala Leu Ile Ser Leu Tyr Arg Asp Val Leu Lys
50 55 60

Ala Lys Glu Asp Pro Ser Ile Arg Tyr Lys Leu Ala Lys Thr Tyr Tyr 65 70 75 80

Gln Arg Gly Asp Ser Lys Ser Ser Leu Leu Tyr Leu Thr Pro Leu Leu 85 90 95

Asn Asp Asn Thr Lys Leu Ala Thr Gln Ala Lys Ile Leu Gln Ile Lys
100 105 110

Asn Leu Ile Gln Leu Asn Asn Phe Gln Glu Ala Ile Ser Val Ala Asn 115 120 125

GIu	130	Leu	Leu	Lys	Ser	Pro 135	Asn	Glu	Gly	Glu	Val 140	Tyr	Asn	Leu	Arg	
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Ile	Asn	Lys	Ala	Arg 165	Glu	Phe	Phe	Ile	Asn 170	Asp	Asn	Val	Ala	Ile 175	Asn	
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Arg																
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acc Thr	gaa Glu	caa Gln	ggc Gly	agc Ser	att Ile	tat Tyr	aac Asn	ata Ile	ggc Gly	ggt Gly	atc Ile	ttg Leu	ggg ggg	gcg Ala	ggt Gly	288

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<213> Pasteurella multocida

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Lys Asn Ile Gln Leu Asn Ala Asn Ile Thr Ile Asn Thr Lys Ser Gly 50 55 60

Phe Val Asn Tyr Gly Thr Leu Ala Ser Ala Gln Asn Leu Thr Ile Asn 65 70 75 80

Thr Glu Gln Gly Ser Ile Tyr Asn Ile Gly Gly Ile Leu Gly Ala Gly
85 90 95

Lys Ser Leu Asn Leu Ser Ala Lys Arg Gly Glu Asn Gln Gly Gly Tyr
100 105 . 110

Leu Ile Asn Gln Gly Lys Ser Leu Leu His Ser Glu Gly Ala Met Asn 115 120 125

Leu Thr Ala Asp Arg Thr Val Tyr Asn Leu Gly Asn Ile Phe Ala Lys 130 135 140

Gly Asp Ala Thr Ile Asn Ala Asn Ala Leu Ile Asn Asp Val Thr Leu 145 150 155 160

Thr Gly Arg Leu Glu Tyr Gln Asp Leu Lys Lys Asp Tyr Thr Arg Tyr
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Ala	Met	Arg	Ala 20	Tyr	Leu	Asp	Lys	Glu 25	Gln	Gly	Trp	His	Thr 30	Ser	Ile	
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	_			_	_	_		_						gaa Glu		192
		_	_	_							_			ggc Gly		240
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act Thr	gca Ala	caa Gln	atc Ile 100	tta Leu	aaa Lys	gat Asp	acg Thr	att Ile 105	gca Ala	gly ggg	gcg Ala	ttt Phe	gat Asp 110	tgg Trp	gca Ala	336
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														ccg Pro 175		528
														gtt Val		576
			cgt Arg				taag	jgggt	ag a	ıaaat	:ggct	t ta	accad	cgcaa	ı	627
actt	aaat	tg a	atgaa	ittta	a to	atco	gacgo	, taa	caaa	tat	ctcg	gcga	ag t	cace	gaagt	687
gact	caac	ca a	aaatt	agca	a to	gaaaa	atcga	aga	attt	cgc	gcgg	gcgg	gta t	gatt	ggttc	747
ggtg	gato	jtc a	aatct	cggg	jc tt	gaaa	agct	cga	agco	gaa	ttta	aago	cg g	gtggc	tacat	807
ggto	gaat	ta a	attaa	aaaa	it to	ggcg	ggto	aat	caac	ggc	atto	catt	gc g	gttt	cttgg	867
ctca	tato	ag d	gtga	tgad	a ca	gaac	gaagt	cac	atct	gtt	gago	ttgt	ga t	gcaa	ggtcg	927
attt	acto	gaa a	attga	cago	g ga	aaca	gcaa	agt	ggg	gat	gaca	ctga	aac a	aaaca	ttcaa	987
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<211> 199 <212> PRT <213> Pasteurella multocida

<400> 67

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Ala Met Arg Ala Tyr Leu Asp Lys Glu Gln Gly Trp His Thr Ser Ile 20 25 30

Ser Asn Lys Gly Ile Asn Gly Val Ser Gly Val Thr Gln Pro Leu Tyr 35 40 45

Phe Asp Ile Asn Asp Ser Ser Thr Asp Val Asn Tyr Leu Asn Glu Gln 50 55 60

Gly Ile Thr Cys Cys Val Asn His Asn Gly Phe Arg Phe Trp Gly Leu 65 70 75 80

Arg Thr Thr Ala Glu Asp Pro Leu Phe Lys Phe Glu Val Tyr Thr Arg 85 90 95

Thr Ala Gln Ile Leu Lys Asp Thr Ile Ala Gly Ala Phe Asp Trp Ala 100 105 110

Val Asp Lys Asp Ile Ser Val Thr Leu Val Lys Asp Ile Ile Glu Ala 115 120 125

Ile Asn Ala Lys Trp Arg Asp Tyr Thr Thr Lys Gly Tyr Leu Ile Gly 130 135 140

Gly Lys Ala Trp Leu Asn Lys Glu Leu Asn Ser Ala Thr Asn Leu Lys 145 150 155

Asp Ala Lys Leu Leu Ile Ser Tyr Asp Tyr His Pro Val Pro Pro Leu 165 170 175

Glu Gln Leu Gly Phe Asn Gln Tyr Ile Ser Asp Glu Tyr Leu Val Asp 180 185 190

Phe Ser Asn Arg Leu Ala Ser 195

<210> 68

<211> 2584

<212> DNA

<213> Pasteurella multocida

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<220>

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<400> 68

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eggtegeeeg ttaatteage	tcaattttgc ca	atgaacgc tta	aacgcca acta	ccgtta 240
ttcacttatc cgtctgaaag	aatatgctga aa	gcattgct ttt	tatcgtg gtga	aaaaat 300
ggaaaaacgt ctattgacca	cacaatttaa tc	aggtgatt gat	aacgttt ggca	agtaat 360
ctaccgcacc ttgaaattat	ccggttttaa ct	taatcatt acg	cagattt cggt	ggtttt 420
tccgctggtg attcaagtga	cacgttattt to	gtogacaa tag	gtgcata tgag	ggtgtt 480
agaatagcga tactttctgt	tggaaaagta aa	ctctttaa tat	aaataga aatc	gcttga 540
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aaatatcttc ttacaatatt	atggtaatta to	aggtaata ccg	statagee atag	attcca 660
gttctatttt gttttgctaa	ataattgatg ag	catttgag gcg	caggtaa atcc	atatct 720
gcaacagaca ttgaaatcat	atccttgccg ta	tttacgag taa	ittgccca ttta	gcacta 780
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taagtgaaat aacgtaattg	atcctcccat tg	ttttacta aat	tatgtct ctga	aactta 1020
tttgttcagg agaaatcatt	_	_	aaa att gaa Lys Ile Glu	
gta aat tat gaa ggt g Val Asn Tyr Glu Gly V 15				
tat gat gct aat caa g Tyr Asp Ala Asn Gln V 30				
cgt tta gcc gtc tgt t Arg Leu Ala Val Cys T 45				-
atg ttc ggt gtc ggt t Met Phe Gly Val Gly S 60			Lys Ala Ser	
tca tta gca ggt gca a Ser Leu Ala Gly Ala L 75				
agt aaa tta ggc ata c Ser Lys Leu Gly Ile P 95	_	_		
cca gaa ggt cat tca t Pro Glu Gly His Ser P 110	•			•
atc gat gtt tta gcg c Ile Asp Val Leu Ala G 125				

											ggt Gly		1503
											caa Gln		1551
											tat Tyr 185		1599
											aat Asn		1647
											gtt Val		1695
											cca Pro		1743
											acc Thr		1791
											gtg Val 265		1839
											cat His		1887
_	_	_	_			_				_	gca Ala		1935
-		_					_	_			aat Asn	_	1983
											gjà aaa		2031
											cag Gln 345		2079
											gat Asp		2127
	_				_			_	_		caa Gln	_	2175
											tca Ser		2223

ggt caa ctt gtt caa atc cgg tcc tac cac gcg tgt ctg caa tac aga 2271 Gly Gln Leu Val Gln Ile Arg Ser Tyr His Ala Cys Leu Gln Tyr Arg 395 400 405 410

cta aca aaa gtg ctt taaaacgttc cggcttacgc cagacatcta gacgattgaa 2326 Leu Thr Lys Val Leu

taatttcaat attgtctccg cacgtaattc aaaggctttg tgtatgtgcg aatgatattc 2386
acaacaaagt tctgcaaaat cttgaattgc gtgaggtaat ttaaagcgct gacataagcg 2446
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<211> 415

<212> PRT

<213> Pasteurella multocida

<400> 69

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Thr Ser Ser Asn Pro Phe Ala Tyr Lys His Tyr Asp Ala Asn Gln Val

Ile Leu Gly Lys Thr Met Ala Glu His Leu Arg Leu Ala Val Cys Tyr
35 40 45

Trp His Thr Phe Cys Trp Thr Gly Asn Asp Met Phe Gly Val Gly Ser

Phe Asp Arg Cys Trp Gln Lys Ala Ser Asp Ser Leu Ala Gly Ala Lys 65 70 75 80

Gln Lys Ala Asp Ile Ala Phe Glu Phe Phe Ser Lys Leu Gly Ile Pro 85 90 95

Tyr Tyr Cys Phe His Asp Val Asp Val Ala Pro Glu Gly His Ser Phe 100 105 110

Lys Glu Tyr Leu Ser Asn Phe Asn Thr Met Ile Asp Val Leu Ala Gln 115 120 125

Lys Gln Glu Glu Thr Gly Val Lys Leu Leu Trp Gly Thr Ala Asn Cys 130 135 140

Phe Thr His Pro Arg Tyr Met Ser Gly Ala Ala Thr Asn Pro Asn Pro 145 150 155 160

Glu Ile Phe Ala Trp Ala Ala Ala Gln Val Phe Thr Ala Met Gly Ala 165 170 175

Thr Gln Arg Leu Gly Gly Glu Asn Tyr Val Leu Trp Gly Gly Arg Glu 180 185

Gly Tyr Glu Thr Leu Leu Asn Thr Asn Leu Lys Gln Glu Arg Glu Gln 195 200 205

Ile Gly Arg Phe Met Gln Met Val Val Glu His Lys Tyr Lys Ile Gly 210 215 Phe Asn Gly Thr Leu Leu Ile Glu Pro Lys Pro Gln Glu Pro Thr Lys 230 235 His Gln Tyr Asp Tyr Asp Val Ala Thr Val Tyr Gly Phe Leu Lys Gln Phe Gly Leu Glu Lys Glu Ile Lys Val Asn Ile Glu Ala Asn His Ala Thr Leu Ala Gly His Thr Phe Gln His Glu Val Ala Met Ala Thr Ala Leu Asp Ile Phe Gly Ser Ile Asp Ala Asn Arg Gly Asp Pro Gln Leu 295 300 Gly Trp Asp Thr Asp Gln Phe Pro Asn Ser Val Glu Glu Asn Thr Leu 310 Val Ile Tyr Glu Ile Leu Lys Ala Gly Gly Phe Thr Thr Gly Gly Phe 330 Asn Phe Asp Ala Lys Ile Arg Arg Gln Ser Thr Asp Pro Tyr Asp Leu Phe His Gly His Ile Gly Ala Ile Asp Val Leu Ala Leu Ser Leu Lys Cys Ala Ala Lys Met Leu Glu Glu Gln Ala Leu Gln Lys Val Val Asn Gln Arg Tyr Ala Gly Trp Thr Ser Ser Leu Gly Gln Leu Val Gln Ile Arg Ser Tyr His Ala Cys Leu Gln Tyr Arg Leu Thr Lys Val Leu <210> 70 <211> 3501 <212> DNA <213> Pasteurella multocida <220> <221> CDS <222> (298)..(1905) <220> <223> yabk <400> 70 gaattcgagg aagggggcgt attacaaatt gaaacggctg cacgtgtagc acaacatqat 60

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								aca Thr								345
								ctg Leu 25								393
								ttt Phe								441
								gtg Val								489
								gtc Val								537
								gtg Val								585
								cct Pro 105								633
								ggc Gly								681
								aat Asn								729
								gtc Val								777
								gtg Val								825
	_	_	_	_		_	•	ttt Phe 185	_			_				873
								gca Ala								921
tgt Cys	ttt Phe 210	acc Thr	agt Ser	ttt Phe	gcg Ala	att Ile 215	gtg Val	ctc Leu	act Thr	tta Leu	ggt Gly 220	ggc Gly	gga Gly	ccg Pro	aaa Lys	969
								tat Tyr								1017
								gcg Ala								1065

	ctg Leu															1113
	tta Leu															1161
	tta Leu 290															1209
	ccg Pro															1257
	acc Thr															1305
ctc Leu	acc Thr	atc Ile	gcc Ala 340	ccc Pro	act Thr	tct Ser	gca Ala	ttg Leu 345	ctc Leu	gct Ala	tta Leu	gta Val	ctg Leu 350	tct Ser	ttt Phe	1353
gcc Ala	tta Leu	tta Leu 355	ttg Leu	ctt Leu	gcc Ala	aga Arg	gaa Glu 360	tta Leu	cat His	tgg Trp	cga Arg	cat His 365	tat Tyr	cgc Arg	agc Ser	1401
	tcc Ser 370															1449
	tta Leu															1497
ttt Phe	tct Ser	cca Pro	tac Tyr	cat His 405	ctt Leu	ttt Phe	ggg ggg	gtt Val	gtg Val 410	gta Val	tgc Cys	tgt Cys	aac Asn	gcg Ala 415	tta Leu	1545
	gct Ala															1593
_	atg Met		_			_	_			_	_	_		_	_ =	1641
tgg Trp	caa Gln 450	cgt Arg	ttt Phe	cga Arg	ttg Leu	att Ile 455	gaa Glu	tgg Trp	cac His	aag Lys	ctt Leu 460	cgt Arg	gcg Ala	cca Pro	atg Met	1689
	tac Tyr	_		_	_	_	_						_			1737
gca Ala	atc Ile	gcg Ala	tta Leu	ttt Phe 485	ggt Gly	cag Gln	gct Ala	gac Asp	ttc Phe 490	aca Thr	tcg Ser	tta Leu	ccg Pro	cat His 495	ttg Leu	1785
	tat Tyr															1833

cga cat cag gaa ccg cgt gat gat taatttaaac ggtgttcagt tttcctataa 1935 Arg His Gln Glu Pro Arg Asp Asp 530 535

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<211> 536

<212> PRT

<213> Pasteurella multocida

<400> 71

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Gly Gly Met Leu Val Ile Val Phe Leu Ser Ala Phe Tyr Ala Phe Ala 20 25 30

Leu Gly Ala Val Phe Ser Leu Pro Phe Ala Arg Ser Trp Thr Ala Leu 35 40 45

Leu Ser Asp Gln Tyr Leu Gln His Val Ile Ile Phe Ser Phe Trp Gln 50 55

Ala Phe Leu Ser Ala Val Leu Ala Val Leu Phe Gly Gly Ile Val Ala 65 70 75 80

Arg Ala Phe Phe Tyr Gln Pro Phe Val Gly Lys Lys Leu Ile Leu Lys

Leu Phe Ser Leu Thr Phe Val Leu Pro Ala Leu Val Ala Ile Phe Gly
100 105 110

Leu Leu Gly Val Tyr Gly Ala Ser Gly Trp Leu Ala Met Leu Ser Gln
115 120 125

Phe Phe Ala Trp Asp Trp Thr Pro Asn Ile Tyr Gly Leu Thr Gly Ile 130 135 140

Leu Leu Ala His Leu Phe Phe Asn Val Pro Leu Ala Cys Arg Leu Phe 145 150 155 160

Leu Gln Gly Leu Gln Ala Ile Pro Val Gln Gln Arg Gln Leu Ala Ala 165 170 175

Gln Leu Asn Leu Arg Gly Trp His Phe Ile Arg Leu Ile Glu Trp Pro 180 185 190

Tyr Leu Arg Gln Gln Leu Leu Pro Ala Phe Thr Leu Ile Phe Met Leu 195 200 205

Cys Phe Thr Ser Phe Ala Ile Val Leu Thr Leu Gly Gly Gly Pro Lys 210 215 220

Tyr Thr Thr Leu Glu Val Ala Ile Tyr Gln Ala Ile Leu Phe Glu Phe 225 235 240

Asp Val Pro Lys Ala Gly Leu Phe Ala Leu Leu Gln Phe Val Phe Cys 245 250 255

Phe Leu Leu Phe Thr Leu Ser Ser Phe Phe Ser Pro Ala Pro Ala Thr 260 265 270

Thr Leu His Ser Gln Pro Thr Trp Phe Ala Pro Gln Ser Tyr Trp Val 275 280 285

Lys Leu Trp Gln Arg Met Ile Ile Val Cys Ala Thr Val Phe Ile Leu 290 295 300

Leu Pro Leu Leu Asn Thr Leu Val Ser Ala Leu Leu Ser Ser Gln Phe 310 315 Phe Thr Leu Trp Leu Gln Pro Gln Leu Trp Lys Ala Leu Gly Tyr Ser 330 Leu Thr Ile Ala Pro Thr Ser Ala Leu Leu Ala Leu Val Leu Ser Phe 345 Ala Leu Leu Leu Ala Arg Glu Leu His Trp Arg His Tyr Arg Ser Leu Ser His Val Ile Leu Asn Ile Gly Ala Thr Ile Leu Ala Ile Pro Thr Leu Val Leu Ala Ile Gly Leu Phe Ile Leu Leu Arg Glu Ile Asp 390 Phe Ser Pro Tyr His Leu Phe Gly Val Val Val Cys Cys Asn Ala Leu Ala Ala Met Pro Phe Val Leu Arg Ile Leu Ala Leu Pro Met His Asn Asn Met Ile Tyr Tyr Glu Lys Leu Cys Gln Ser Leu Asn Leu Arg Gly Trp Gln Arg Phe Arg Leu Ile Glu Trp His Lys Leu Arg Ala Pro Met Lys Tyr Ala Phe Ala Leu Ala Cys Ala Leu Ser Leu Gly Asp Phe Thr Ala Ile Ala Leu Phe Gly Gln Ala Asp Phe Thr Ser Leu Pro His Leu 490 Leu Tyr Gln Gln Leu Gly His Tyr Arg Ser Gln Glu Ala Ala Val Thr Ala Phe Ile Leu Leu Val Phe Cys Leu Ser Val Phe Met Ile Ile Glu 515 520 Arg His Gln Glu Pro Arg Asp Asp <210> 72 <211> 3182 <212> DNA <213> Pasteurella multocida

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											ttt Phe				cca Pro	1699
											att .Ile					1747
											tca Ser 80					1795
											atg Met					1843
											gat Asp					1891
											att Ile					1939
atg Met	ttt Phe	gtt Val 135	gca Ala	gtt Val	cta Leu	ctt Leu	gct Ala 140	acg Thr	atg Met	tca Ser	ggt Gly	att Ile 145	atc Ile	ggt Gly	gga Gly	1987
											caa Gln 160					2035
											gta Val					2083
											att Ile					2131
											gca Ala					2179
_	-	_	_	_			_		_	_	att Ile	_		_		2227
											agc Ser 240					2275
											cat His					2323
											att Ile					2371
											gtt Val					2419

gca Ala	gca Ala	ttc Phe 295	tat Tyr	cga Arg	aaa Lys	gaa Glu	tta Leu 300	aat Asn	ttc Phe	aaa Lys	ata Ile	gta Val 305	caa Gln	gaa Glu	tca Ser	2467
cta Leu	aaa Lys 310	cat His	aca Thr	atc Ile	aat Asn	act Thr 315	gtt Val	ggt Gly	atg Met	ata Ile	atc Ile 320	tgg Trp	gtc Val	ggc Gly	att Ile	2515
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														tat Tyr 355		2611
														tta Leu		2659
														gcg Ala		2707
														ggt Gly		2755
														cgc Arg		2803
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gaac	caagt	gt t	atac	caaç	ja ag	cgaa	ıtcca	aca	ggtg	jaag	tggt	gato	gg t	tatgg	tgggt	3039
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ttaa	aaaa	cc ç	gtett	agco	jt go	aaat	caaa	tat	attg	jatt	caca	agat	gt	ggaaa	ccaaa	3159
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<210> 73

<211> 422

<212> PRT

<213> Pasteurella multocida

<400> 73

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Leu Tyr Lys Ile Ser Gly Gly Ile Ile Met Ile Ser Ala Phe Gly Ile 20 25 30

Gly Ile Gly Thr Leu Ile Ile Phe Leu Met Met Ile Ser Leu Leu Phe 35 40 45

Ile Gly Met Pro Leu Gly Phe Leu Thr Gly Leu Ile Ala Leu Val Ile Ser Tyr Leu Trp Phe Asp Thr Thr Ala Ile Met Gln Met Ile Ala Ser Arg Val Thr Asp Phe Thr Ser Ser Tyr Thr Phe Val Ala Val Pro Met Phe Val Leu Met Ala Thr Leu Leu Asp Lys Thr Gly Ile Ala Arg Asp 105 Leu Tyr Asn Ala Met Arg Val Ile Gly Gly Arg Leu Arg Gly Gly Ile 120 Ala Ile Gln Ser Met Phe Val Ala Val Leu Leu Ala Thr Met Ser Gly Ile Ile Gly Gly Glu Thr Val Leu Leu Gly Met Leu Ala Leu Pro Gln 155 Met Leu Arg Leu Gly Tyr Asn Lys Asn Leu Ala Ile Gly Thr Val Val 170 Ala Gly Gly Ala Leu Gly Thr Met Val Pro Pro Ser Ile Val Leu Ile 180 185 Ile Tyr Gly Met Thr Ala Asn Val Ser Ile Gly Glu Leu Phe Leu Ala 200 Ala Ile Pro Ala Ser Leu Leu Ser Thr Phe Tyr Ile Leu Tyr Ile Leu Val Leu Cys Tyr Phe Lys Pro Ser Tyr Gly Pro Ala Met Pro Ser Ser Glu Asn His Thr Leu Thr Lys Glu Asp Ile Lys Lys Ile Ile His Asp Ile Ala Ile Pro Val Ala Ile Ala Thr Trp Ile Leu Gly Ser Ile 265 Tyr Gly Gly Ile Ala Ser Ile Thr Glu Ser Ala Cys Val Gly Val Val Gly Val Ile Leu Ala Ala Phe Tyr Arg Lys Glu Leu Asn Phe Lys Ile 295 Val Gln Glu Ser Leu Lys His Thr Ile Asn Thr Val Gly Met Ile Ile Trp Val Gly Ile Gly Ala Thr Met Ile Ile Gly Ile Tyr Asn Leu Met Gly Gly Asp Arg Phe Ile Ala Asn Leu Phe Ala Ser Leu Asp Ala Ser Pro Ile Tyr Thr Ile Ile Ile Met Met Val Ile Leu Leu Ile Leu Gly 360 Met Phe Leu Asp Trp Ile Gly Val Ala Met Leu Thr Phe Leu Lys Thr

Ser Lys Ala Thr ile Asn Leu Cys Phe Asp Ile Val Arg Tyr Ser Ile 390 Trp Arg Gly Pro Ser Phe His Ser Thr Asn Val His Arg Gly Thr Phe 410 Val Gly Arg Gly Thr Phe 420 <?10> 74 <211> 2787 <212> DNA <213> Pasteurella multocida <220> <221> CDS <222> (463)..(936) <220> <223> yhcJ <400> 74 gttaacacac catgattaat gatgccggtt gaagccactg caacgtaatc gaattgtccq 60 gcatacaaag caagaatgtt ggccagtgtg tcatgcatcg cattggcagc atcagcttgt 120 ggcgttgcaa tetgttggcg ttgttetatt ttgccgtetg ttacaatage eqaqecaatt 180 tttgttccac caatatctaa tgctaaacag cgcatagget eteettetgt gatgaettat 240 tttgccgatt tgacggcatc ggcaaaccag cttacgatat gttcgaggcg agtcagcgca 300 gatectaegg tgacagagta agcaccaate teaattgegg ttttegecaa ttetggggtg 360 ttatagcgcc cttctgccat cactcggcag ccagcagcat tcaaatcttt gactaactga 420 taatccggtt cagctggaat ttcaccgcca gtataaccag ac atg gtg cta cca Met Val Leu Pro ata att tet ace eet aag ttg tgg caa tae ate eet tet tea aaa tta Ile Ile Ser Thr Pro Lys Leu Trp Gln Tyr Ile Pro Ser Ser Lys Leu 10 gaa caa too goo atg got aaa caa cot aat tot ttg att ogt tta ata Glu Gln Ser Ala Met Ala Lys Gln Pro Asn Ser Leu Ile Arg Leu Ile 25 atg gct tca cgt gta gtt gga cgg acg cga tcg gta cca tca aaa gca Met Ala Ser Arg Val Val Gly Arg Thr Arg Ser Val Pro Ser Lys Ala 40 ata ata tcg gcg cct gct gcg gct aac tct tca atg tct tgt aaa aat 666 Ile Ile Ser Ala Pro Ala Ala Ala Asn Ser Ser Met Ser Cys Lys Asn 55 60 ggg cta ata cga acg gga ctg tca ggt aaa tcg cgt tta acg ata cca 714 Gly Leu Ile Arg Thr Gly Leu Ser Gly Lys Ser Arg Leu Thr Ile Pro ata atc ggt aca ttg acg acg tta cgc gtg gct ttt aaa ttt tcg atc 762 Ile Ile Gly Thr Leu Thr Thr Leu Arg Val Ala Phe Lys Phe Ser Ile

85	90	95	100	
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	t gcg att aag cca tat p Ala Ile Lys Pro Tyr 5 140			906
	t ttt gac ata tta act r Phe Asp Ile Leu Thr 155		atttatc aaaagaagat	956
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<210> 75

<211> 158

<212> PRT

<213> Pasteurella multocida

<400> 75

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Ser Ser Lys Leu Glu Gln Ser Ala Met Ala Lys Gln Pro Asn Ser Leu 20 25 30

Ile Arg Leu Ile Met Ala Ser Arg Val Val Gly Arg Thr Arg Ser Val
35 40 45

Pro Ser Lys Ala Ile Ile Ser Ala Pro Ala Ala Ala Asn Ser Ser Met 50 55 60

Ser Cys Lys Asn Gly Leu Ile Arg Thr Gly Leu Ser Gly Lys Ser Arg 65 70 75 80

Leu Thr Ile Pro Ile Ile Gly Thr Leu Thr Thr Leu Arg Val Ala Phe
85 90 95

Lys Phe Ser Ile Pro Ser Ile Arg Asn Pro Ala Ala Pro Pro Ile Thr

Asp Ala Cys Ala Met Ala Ala Thr Ile Ser Gly Glu Ser Ile Gly Pro 115 120 125

Leu Ser Thr Gly Trp Gln Asp Ala Ile Lys Pro Tyr Leu 1le Cys Ser 130 140

Lys Thr Cys Gly Cys Asp Ser Phe Asp Ile Leu Thr Pro Val 145 150 155

<210> 76

<211> 2787

<212> DNA

<213> Pasteurella multocida

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<220> <223> yia0

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gca tct tta tgt Ala Ser Leu Cys 10				
gat ctt aaa ttc Asp Leu Lys Phe 25			Ser Asn Glu	
gca gta gaa ttc Ala Val Glu Phe				
att gat gtg gct Ile Asp Val Ala 60				
atg att aaa caa Met Ile Lys Gln 75		y Ala Leu Asp		
tca gca cgt ttc Ser Ala Arg Phe 90				
cct tat atg att Pro Tyr Met Ile 105			Lys Ala Leu	
aca aaa ttt ggt Thr Lys Phe Gly				
gta caa gtg tta Val Gln Val Leu 140				
aac cgt gca atc Asn Arg Ala Ile 155		u Asp Met Lys		
gta cct aac gcg Val Pro Asn Ala 170	gca acc aac ct Ala Thr Asn Let 175	t gct tat gca u Ala Tyr Ala	aaa tac gtg Lys Tyr Val 180	ggt gca 2500 Gly Ala
gcg cca aca cca Ala Pro Thr Pro 185			Leu Ala Leu	
aac tct gtg gat Asn Ser Val Asp		_		-
aaa ttc tat gaa Lys Phe Tyr Glu 220				

aat gac caa ctt tac tta atc agt aac gat acg ttg gca gat tta cca 2692
Asn Asp Gln Leu Tyr Leu Ile Ser Asn Asp Thr Leu Ala Asp Leu Pro
235

gaa gat tta caa aaa gtg gtt aaa gat gca gca gcg aaa gcc gct gaa
Glu Asp Leu Gln Lys Val Val Lys Asp Ala Ala Ala Lys Ala Ala Glu
2740

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Tyr His Thr Lys Leu Phe Val Asp Gly Glu Asn Ser Leu Val Glu
2787

<210> 77

<211> 279

<212> PRT

<213> Pasteurella multocida

<400> 77

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Ala Ser Val Phe Ala Ala Asp Tyr Asp Leu Lys Phe Gly Met Val Ala
20 25 30

Gly Pro Ser Ser Asn Glu Tyr Lys Ala Val Glu Phe Phe Ala Lys Glu 35 40 45

Val Lys Glu Lys Ser Asn Gly Lys Ile Asp Val Ala Ile Phe Pro Ser 50 55 60

Ser Gln Leu Gly Asp Asp Arg Val Met Ile Lys Gln Leu Lys Asp Gly 65 70 75 80

Ala Leu Asp Phe Thr Leu Gly Glu Ser Ala Arg Phe Gln Ile Tyr Phe
85 90 95

Pro Glu Ala Glu Val Phe Ala Leu Pro Tyr Met Ile Pro Asn Phe Glu 100 105 110

Thr Ser Lys Lys Ala Leu Leu Asp Thr Lys Phe Gly Gln Gly Leu Leu 115 120 125

Lys Lys Ile Asp Lys Glu Leu Asn Val Gln Val Leu Ser Val Ala Tyr 130 135 140

Asn Gly Thr Arg Gln Thr Thr Ser Asn Arg Ala Ile Asn Ser Ile Glu 145 150 155 160

Asp Met Lys Gly Leu Lys Leu Arg Val Pro Asn Ala Ala Thr Asn Leu

Ala Tyr Ala Lys Tyr Val Gly Ala Ala Pro Thr Pro Met Ala Phe Ser 180 185 190

Glu Val Tyr Leu Ala Leu Gln Thr Asn Ser Val Asp Gly Gln Glu Asn 195 200 205

Pro Leu Pro Thr Ile Gln Ala Gln Lys Phe Tyr Glu Val Gln Lys Tyr 210 215 220

Leu Ala Leu Thr Asn His Ile Leu Asn Asp Gln Leu Tyr Leu Ile Ser 225 230 235

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1045

ggg caa att cca gtg aat cca aaa acc ggt gaa gtg cca gcg gat atc

Gly Gln Ile Pro Val Asn Pro Lys Thr Gly Glu Val Pro Ala Asp Ile

20

15

35 40 45 gta gca caa gca cgt caa tcq tta gaa aac gtg aaa gcg att gtg gaa 1093 Val Ala Gln Ala Arg Gln Ser Leu Glu Asn Val Lys Ala Ile Val Glu caa gcg gga tta caa gtc gca aat atc gtg aaa acc acg gtg ttt gtg 1141 Gln Ala Gly Leu Gln Val Ala Asn Ile Val Lys Thr Thr Val Phe Val aaa gat tta aat gac ttt gca g g gtc aat gcg gag tat gaa cgt ttc 1189 Lys Asp Leu Asn Asp Phe Ala Ala Val Asn Ala Glu Tyr Glu Arg Phe ttt aaa gag aac aat cac cct agc ttc cct gct cgt tca tgt gtg gaa 1237 Phe Lys Glu Asn Asn His Pro Ser Phe Pro Ala Arg Ser Cys Val Glu 95 100 105 gtg gca cgt ttg ccg aaa gat gtg ggg att gaa atc gag gca atc gct 1285 Val Ala Arg Leu Pro Lys Asp Val Gly Ile Glu Ile Glu Ala Ile Ala 120 gta aaa gcc taatgaatag cttgcattta tcttagtcgt agcaaaacaa 1334 Val Lys Ala tototttttca ottgototot toaaagcaag ttgataagtg atttttattg ggogttnttc 1394 tattgatago caaaaacgoo otttactgat agagaataaa otatgcaaaa toaaqtcato 1454 gagattetae aatacegttt aaaaceacaa teaggacaaa egttteacea aattatgegt 1514 gagatcagtg ttccactcca taaacaacat gggattgatg tcattgcgta tggaaattca 1574 ttacatgata ttgacageta ttatttaate egtgeatttg agacagaaac caaattgeaa 1634 cagcageteg atgettttta tgccagtgat gattggegtg atggaccaag agaaagtate 1694 attogootga ttgaaagcag tttaaaatog gtgatoatgo toocgacaca ggcaatocat 1754 gcactacgca accattatcc tcaataaaat caacaaccgc acccaatcag tgcggtcatt 1814 ttttcttact ttttcagtgc taagggaaaa acaacgatag tggacgttgt ttaatcaatt 1874 tecaaacaca ttgegegata teacaecaae teteaattte tgtttetaaa gaaegeageg 1934 caacccataa cgcgataaag aaactgacaa tcaaattcac cataccaatc aataacacqa 1994 acactaaacc ttgtaagaac atctgccaag taaacgcgcc actgatcgcc atatagccca 2054 aattogoaga agaaaacgoo acatggogaa tatotaacgg taaattaago aaataccoga 2114 ctaaccccgt taaaccaagc aataaaccaa aacacagatt tcccataatc gaaccgtaat 2174 tatcatgoca gtattotgoa aatttgtago goatattaog ggtoaacaga oggogtaaaa 2234 tagggtgatt tettagtege attittaagt teaaataatt aetaegatta teaaaataae 2294 cagaaataat cccagaaaag aataaccaga aacccgcaat ggcggcaaac cataaggacc 2354 ctttcatcgg atcaagggat ttttgttggt aggcaatctc cgcgtcactc aataaaggtq 2414 taccaacata atgttgatag cctagegeaa geaaacaage cacagaaate getaaagtga 2474

cattacccaa gactgccact gtttgcgagc gaaacacatc gatcagcagt tgagctaatt 2534 gcagattaac ggatttgcct tgtccattat ccaccgtttc tgcaaaacgt gctgca 2590

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<211> 129

<212> PRT

<213> Pasteurella multocida

:400> 79

Met Thr Lys Val Ile His Thr Asp Asn Ala Pro Ala Ala Ile Gly Pro 1 5 10 15

Tyr Val Gln Ala Val Asp Leu Gly Asn Met Leu Leu Thr Ser Gly Gln
20 25 30

Ile Pro Val Asn Pro Lys Thr Gly Glu Val Pro Ala Asp Ile Val Ala
35 40 45

Gln Ala Arg Gln Ser Leu Glu Asn Val Lys Ala Ile Val Glu Gln Ala 50 55 60

Gly Leu Gln Val Ala Asn Ile Val Lys Thr Thr Val Phe Val Lys Asp 65 70 75 80

Leu Asn Asp Phe Ala Ala Val Asn Ala Glu Tyr Glu Arg Phe Phe Lys
85 90 95

Glu Asn Asn His Pro Ser Phe Pro Ala Arg Ser Cys Val Glu Val Ala
100 105 110

Arg Leu Pro Lys Asp Val Gly Ile Glu Ile Glu Ala Ile Ala Val Lys 115 120 125

Ala

<210> 80

<211> 6642

<212> DNA

<213> Pasteurella multocida

<220>

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<222> (463)..(1884)

<220>

<223> yleA

<400> 80

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ggg	aatti	ca a	aaaga	aaat	ct c	ttti	ttag	c tag	gcca	gcat	aggi	ttcaa	aga (ctgta	aaaata	420
gtca	agtca	aca 1	tttt	catag	gg ti	caact	tgaat	t tt	ttta	aacg		_		caa a Gln 1		474
							tgt Cys									522
	_	_	_				agt Ser								_	570
							tta Leu									618
							ttc Phe 60									666
							ctc Leu									714
							att Ile									762
							tta Leu									810
							tca Ser									858
							cca Pro 140									906
							ggc Gly									954
							gaa Glu									1002
							ttg Leu									1050
							aac Asn									1098
							gaa Glu 220									1146
gat	ggt	att	gac	cgt	tta	cgt	ttt	acc	acc	agt	cac	cca	att	gag	ttc	1194

Asp	Gly 230	Ile	Asp	Arg	Leu	Arg 235	Phe	Thr	Thr	Ser	His 240	Pro	Ile	Glu	Phe	
act Thr 245	gat Asp	gac Asp	att Ile	att Ile	gat Asp 250	gtg Val	tac Tyr	cgt Arg	gat Asp	acg Thr 255	cca Pro	gag Glu	ttg Leu	gtg Val	agt Ser 260	1242
			tta Leu													1290
			aat Asn 280													1338
tta Leu	aga Arg	gcg Ala 295	gtg Val	cgt Arg	cca Pro	gag Glu	att Ile 300	caa Gln	att Ile	agc Ser	tca Ser	gat Asp 305	ttt Phe	att Ile	gtc Val	1386
			ggc Gly													1434
			gta Val													1482
-			acg Thr		_	_	_	_		_	_			_	_	1530
			caa Gln 360													1578
			ttt Phe													1626
	_		ccc Pro	_			_		_	_				_		1674
			cgt Arg													1722
			gat Asp													1770
			gtt Val 440													1818
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<212> PRT

<213> Pasteurella multocida

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Glu Leu Thr Glu Ile Pro Glu Glu Ala Asp Val Leu Leu Leu Asn Thr 35 40 45

Cys Ser Ile Arg Glu Lys Ala Gln Glu Lys Val Phe His Gln Leu Gly 50 60

Arg Trp Lys Glu Leu Lys Lys His Lys Pro Gly Leu Val Ile Gly Val 65 70 75 80

Gly Gly Cys Val Ala Ser Gln Glu Gly Glu His Ile Arg Thr Arg Ala 85 90 95

Pro Tyr Val Asp Ile Ile Phe Gly Pro Gln Thr Leu His Arg Leu Pro 100 105 110

Glu Met Ile Asn Gln Ile Arg Gly Gly Lys Ser Ser Val Val Asp Val 115 120 125

Ser Phe Pro Glu Ile Glu Lys Phe Asp Arg Leu Pro Glu Pro Arg Ala 130 135

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Pro	Val	Asp	Asp 180	Val	Leu	Phe	Glu	Ile 185	Ala	Gln	Leu	Ala	Glu 190	Gln	Gly
Val	Arg	Glu 195	Val	Asn	Leu	Leu	Gly 200	Gln	Asn	Val	Asn	Ala 205	Tyr	Arg	Gly
Ala	Thr 210	His	Asp	Asp	Gly	Ile 215	Cys	Thr	Phe	Ala	Glu 220	Leu	Leu	Arg	Leu
Val 225	Ala	Ala	Ile	Asp	Gly 230	Ile	Asp	Arg	Leu	Arg 235	Phe	Thr	Thr	Ser	His 240
Pro	Ile	Glu	Phe	Thr 245	Asp	Asp	Ile	Ile	Asp 250	Val	Tyr	Arg	Asp	Thr 255	Pro
Glu	Leu	Val	Ser 260	Phe	Leu	His	Leu	Pro 265	Val	Gln	Ser	Gly	Ser 270	Asp	Arg
Val	Leu	Ser 275	Met	Met	Lys	Arg	Asn 280	His	Thr	Ala	Leu	Glu 285	Tyr	Lys	Ser
Ile	Ile 290	Arg	Lys	Leu	Arg	Ala 295	Val	Arg	Pro	Glu	Ile 300	Gln	Ile	Ser	Ser
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Val	Thr	Glu 355	Glu	Glu	Lys	Lys	Gln 360	Arg	Leu	Tyr	Val	Leu 365	Gln	Gln	Arg
Ile	Asn 370	Asn	Gln	Ala	Ala	Gln 375	Phe	Ser	Arg	Ala	Met 380	Leu	Gly	Thr	Glu
Gln 385	Arg	Val	Leu	Val	Glu 390	Gly	Pro	Ser	Lys •	Lys 395	Asp	Leu	Met	Glu	Leu 400
Thr	Gly	Arg	Thr	Glu 405	Thr	Asn	Arg	Ile	Val 410	Asn	Phe	Val	Gly	Thr 415	Pro
Asp	Met	Ile	Gly 420	Lys	Phe	Val	Asp	Ile 425	Lys	Ile	Thr	Asp	Val 430	Phe	Thr
Asn	Ser	Leu 435	Arg	Gly	Glu	Val	Val 440	Arg	Thr	Glu	Glu	Gln 445	Met	Gly	Leu
Arg	Val 450	Val	Gln	Ser	Pro	Gln 455	Met	Val	Ile	Asn	Arg 460	Thr	Arg	Lys	Glu
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135 145 140 tgt gat gtg tgt tat cgc gtc tgt ccg ctg att aat aaa gcg att acg 895 Cys Asp Val Cys Tyr Arg Val Cys Pro Leu Ile Asn Lys Ala Ile Thr 160 tta gtg atg cat cgt aat gag cgt acg ggt aag cac gcc gtc ttt atc 943 Leu Val Met His Arg Asn Glu Arg Thr Gly Lys His Ala Val Phe Ile 165 170 175 cca aca gtg cat tcc gaa gcc tgt aca gga tgt ggc aaa tgt gaa gaa 991 Pro Thr Val His Ser Glu Ala Cys Thr Gly Cys Gly Lys Cys Glu Glu 180 185 gct tgc gtt cta gaa gaa gcg gca atc aaa gtg tta ccg atg gca tta 1039 Ala Cys Val Leu Glu Glu Ala Ala Ile Lys Val Leu Pro Met Ala Leu 200 205 210 gcg aaa ggc atg tta ggt aaa cat tac cgt tta ggt tgg gaa gag aaa 1087 Ala Lys Gly Met Leu Gly Lys His Tyr Arg Leu Gly Trp Glu Glu Lys gaa aaa gcc ggg cat tcc ctt gcg cca gaa ggc att att tct ctc ccg 1135 Glu Lys Ala Gly His Ser Leu Ala Pro Glu Gly Ile Ile Ser Leu Pro act cgg tta ccg gag agc ttg taatggcaaa ttcaccaaaa tatgcgggta 1186 Thr Arg Leu Pro Glu Ser Leu aagaagcacg agaaaagtta ggctggtggt acgccaatcg ctttttgttc tggcgacgtt 1246 taacccagct gagtattctt gccatgtttt taagcggacc ttattttggg gtgtggatct 1306 ttatggcaga aagtctcgcg accggtttta tgcctaccat gaccgcgttg ttgggtgccc 1426 tgattgtggt ggtgctttat gccattttag ggagtcgcgt tttctgtgct tgggtttgtc 1486 cgttaaacat cgtgacagat gcgtccgctt ggctcagacg taaattagaa attcgtcaat 1546 cggcaaaact cccacgaagt ttacgctatg cgatcttagt gatgattttg ttaggcagtg 1606 cgctaagcgg gttattactt tgggaatggc tcaatccggt tgcagcacta ggtcgtgcgt 1666 taatttacgg tttcggtgcg acagtttggc ttgttcttgc ggtgttttta tttgatttat 1726 ttattgtcga gcatggttgg tgtgggcatt tatgcccaat aggtgcagcc tatggtgtga 1786 ttggtgcgaa aggacttttt cgtatcaaag ttgagcatcg ccaacaatgt gataattgca 1846 tggattgcta taacgtctgt ccagaaccac aagtgttacg cgatccatta catgcaaaaa 1906 agagtgaaag cccacttgtg ctttcaaaag attgtatcag ctgtggacgt tgtatcgacg 1966 tttgccctga aaaagtattt atttttacaa cacgatttaa tcattcagtt aatcattcgg 2026 gggagtgatc aaaatgaaaa aaacaatgct aattttgacc gcactttttg ccttcacggt 2086 caatgccaat gaagtcaaag tgggcaaaag tttacaagat tcacctgaaa atatcgcgcc 2146 agcettecae aatacaecaa aagaaagtgg ettggegeeg ttaaactatg tgaaccaace 2206

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<213> Pasteurella multocida

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Leu Gln Gln Lys Gln Ser Leu Ala Arg Glu Gly Val Ala Leu Arg Pro
35 40 45

Pro Phe Ala Leu Glu Asn Glu Lys Ala Phe Ser Ala Ala Cys Ile Arg 50 55 60

Cys Gly Gln Cys Val Gln Ala Cys Pro His Glu Met Leu His Leu Ala 65 70 75 80

Ser Leu Ile Ser Pro Met Giu Ala Gly Thr Pro Tyr Phe Ile Ala Arg 85 90 95

Asp Lys Pro Cys Glu Met Cys Val Asp Ile Pro Cys Ala Lys Ala Cys 100 105 110

Pro Thr Gly Ala Leu Asp Asn Gln Ala Thr Glu Ile Asp Asp Ala Arg 115 120 125

Met Gly Leu Ala Val Leu Leu Asp His Glu Thr Cys Leu Asn Trp Gln 130 135

Gly Leu Arg Cys Asp Val Cys Tyr Arg Val Cys Pro Leu Ile Asn Lys 145 150 155 160

Ala Ile Thr Leu Val Met His Arg Asn Glu Arg Thr Gly Lys His Ala 165 170 175

Val Phe Ile Pro Thr Val His Ser Glu Ala Cys Thr Gly Cys Gly Lys 180 185 190

Cys Glu Glu Ala Cys Val Leu Glu Glu Ala Ala Ile Lys Val Leu Pro 195 200 205

Met Ala Leu Ala Lys Gly Met Leu Gly Lys His Tyr Arg Leu Gly Trp 210 215 220

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Tyr Val Thr Ile Ser Thr Leu Asn Arg Val
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35 40 45

Ser Val Phe Ile Leu Ala Cys Phe Phe Tyr Tyr Arg Ala Glu Leu Thr 50 55 60

Ser Ser Gly Ala Gly Val Gln Ser Val Ala Met Leu Pro Ser Ser 65 70 75 80

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cgt Arg	gtt Val	ttt Phe 35	tcg Ser	cgt Arg	gat Asp	gag Glu	aag Lys 40	aaa Lys	caa Gln	gat Asp	gac Asp	atg Met 45	cgg Arg	aaa Lys	aaa Lys	144
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tta Leu	tta Leu	tct Ser	gtc Val	cca Pro	aat Asn	cac His	cct Pro	att Ile	tcc Ser	att Ile	ata Ile	ggt Gly	acg Thr	cgt Arg	cat His	768

245 250 255 gga gag aaa gca ttc gaa gct tta tta agc cgt gaa gaa atg gtt cat 816 Gly Glu Lys Ala Phe Glu Ala Leu Leu Ser Arg Glu Glu Met Val His 265 gca att aat gaa ggt aat tat tat cgc atc cca gcc gat caa cgc aqt 864 Ala Ile Asn Glu Gly Asn Tyr Tyr Arg Ile Pro Ala Asp Gln Arg Ser 280 tta aat tac agt aaa tat gtc gaa aaa ggg gaa cca aaa att acc gaa 912 Leu Asn Tyr Ser Lys Tyr Val Glu Lys Gly Glu Pro Lys Ile Thr Glu gtc acc gac tac aac tca cat aat act gag cgt ttg act gtc aag gaa Val Thr Asp Tyr Asn Ser His Asn Thr Glu Arg Leu Thr Val Lys Glu 305 310 atg aag cag tta ctg ctt aaa ctt gaa ttc ata cag aaa atg att gag Met Lys Gln Leu Leu Lys Leu Glu Phe Ile Gln Lys Met Ile Glu 325 ggt gaa tac atc tca ccg gag gta ta 1034 Gly Glu Tyr Ile Ser Pro Glu Val 340 <210> 101 <211> 344 <212> PRT <213> Pasteurella multocida <400> 101 Met Phe Lys Asn Lys Thr Leu Leu Ile Thr Gly Gly Thr Gly Ser Phe Gly Asn Ala Val Leu Lys Arg Phe Leu Glu Thr Asp Ile Arg Glu Ile Arg Val Phe Ser Arg Asp Glu Lys Lys Gln Asp Asp Met Arg Lys Lys Tyr Asn Asp Ala Lys Leu Lys Phe Tyr Ile Gly Asp Val Arg Asp Tyr Asp Ser Ile Leu Asn Ala Ser Arg Gly Val Asp Tyr Ile Tyr His Ala Ala Ala Leu Lys Gln Val Pro Ser Cys Glu Phe Tyr Pro Leu Glu Ala Val Lys Thr Asn Ile Leu Gly Thr Ala Asn Val Leu Glu Ala Ala Ile Gln Asn Gln Ile Lys Arg Val Val Cys Leu Ser Thr Asp Lys Ala Val Tyr Pro Ile Asn Ala Met Gly Ile Ser Lys Ala Met Met Glu Lys Val 135 Ile Ile Ala Lys Ser Arg Asn Leu Glu Gly Thr Pro Thr Thr Ile Cys 150

Cys Thr Arg Tyr Gly Asn Val Met Ala Ser Arg Gly Ser Val Ile Pro Leu Phe Val Asp Gln Ile Arg Gln Gly Lys Pro Phe Thr Ile Thr Asp 185 Pro Glu Met Thr Arg Phe Met Met Thr Leu Glu Asp Ala Val Asp Leu 200 Val Leu Tyr Ala Phe Lys Asn Gly Gln Asn Gly Asp Val Phe Val Gln 215 Lys Ala Pro Ala Ala Thr Ile Gly Thr Leu Ala Lys Ala Ile Thr Glu Leu Leu Ser Val Pro Asn His Pro Ile Ser Ile Ile Gly Thr Arg His 245 Gly Glu Lys Ala Phe Glu Ala Leu Leu Ser Arg Glu Glu Met Val His Ala Ile Asn Glu Gly Asn Tyr Tyr Arg Ile Pro Ala Asp Gln Arg Ser Leu Asn Tyr Ser Lys Tyr Val Glu Lys Gly Glu Pro Lys Ile Thr Glu Val Thr Asp Tyr Asn Ser His Asn Thr Glu Arg Leu Thr Val Lys Glu Met Lys Gln Leu Leu Lys Leu Glu Phe Ile Gln Lys Met Ile Glu 330 Gly Glu Tyr Ile Ser Pro Glu Val 340 <210> 102 <211> 4931 <212> DNA <213> Pasteurella multocida <220> <223> fhaB2 <220> <221> CDS <222> (1)..(4929) <220> <221> misc feature <222> 1632 <223> Xaa = any or unknown amino acid atg aac aaa aat cgt tac aaa ctc att ttt agt caa gtc aaa ggt tgt 48 Met Asn Lys Asn Arg Tyr Lys Leu Ile Phe Ser Gln Val Lys Gly Cys 15 ctc gtt cct gtg gca gaa tgt att aac tca gct att agc aat ggt tca Leu Val Pro Val Ala Glu Cys Ile Asn Ser Ala Ile Ser Asn Gly Ser

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ctt Leu 305	att Ile	gcg Ala	ggt Gly	tcc Ser	agt Ser 310	gaa Glu	tat Tyr	gat Asp	tta Leu	agc Ser 315	aaa Lys	cat His	gag Glu	ctg Leu	aaa Lys 320	960
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tct Ser	agt Ser	aca Thr	ggc Gly 340	gca Ala	atg Met	cat His	ggt Gly	aaa Lys 345	aat Asn	att Ile	aag Lys	ttg Leu	att Ile 350	gtg Val	aca Thr	1056
gat Asp	aaa Lys	ggt Gly 355	gca Ala	ggc Gly	gta Val	aaa Lys	cat His 360	gat Asp	gga Gly	att Ile	att Ile	ttg Leu 365	tct Ser	gaa Glu	aat Asn	1104
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att Ile 385	cag Gln	caa Gln	aca Thr	gtg Val	gta Val 390	aaa Lys	aaa Lys	gac Asp	cga Arg	aat Asn 395	att Ile	cga Arg	gcc Ala	aag Lys	aaa Lys 400	1200
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gcc Ala	ctg Leu 450	tct Ser	att Ile	gag Glu	caa Gln	aat Asn 455	gcg Ala	aag Lys	ctc Leu	gtc Val	gct Ala 460	aaa Lys	aag Lys	ata Ile	gat Asp	1392
gtg Val 465	gca Ala	aca Thr	gaa Glu	act Thr	cta Leu 470	act Thr	aat Asn	gct Ala	ggg Gly	cgt Arg 475	att Ile	tat Tyr	ggt Gly	cga Arg	gag Glu 480	1440
gtt Val	aag Lys	ctt Leu	gac Asp	act Thr 485	aat Asn	aat Asn	ttg Leu	att Ile	aat Asn 490	gat Asp	aaa Lys	gaa Glu	att Ile	tat Tyr 495	gct Ala	1488
gaa Glu	cgg Arg	aaa Lys	ttg Leu 500	agt Ser	att Ile	ttg Leu	acg Thr	aaa Lys 505	gga Gly	aaa Lys	gat Asp	ctt Leu	gaa Glu 510	att Ile	att Ile	1536
caa Gln	gat Asp	aga Arg 515	tat Tyr	ttg Leu	tct Ser	cca Pro	ctg Leu 520	atg Met	cgc Arg	gta Val	aaa Lys	agt Ser 525	agt Ser	gtc Val	cgc Arg	1584

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	tta Leu 530															1632
ctt Leu 545	agt Ser	gca Ala	cag Gln	ttt Phe	aag Lys 550	cct Pro	ggt Gly	ttt Phe	gtg Val	aat Asn 555	aag Lys	gga Gly	ctc Leu	att Ile	gaa Glu 560	1680
agt Ser	gcg Ala	ggg Gly	agt Ser	gca Ala 565	gaa Glu	tta Leu	act Thr	ttt Phe	aaa Lys 570	gaa Glu	aaa Lys	acc Thr	agt Ser	ttt Phe 575	tta Leu	1728
aca Thr	gag Glu	ggc Gly	aat Asn 580	aat Asn	ttt Phe	att Ile	aga Arg	gct Ala 585	aaa Lys	gat Asp	gcg Ala	tta Leu	act Thr 590	att Ile	aac Asn	1776
gcc Ala	caa Gln	aat Asn 595	att Ile	gaa Glu	att Ile	gat Asp	aaa Lys 600	aat Asn	caa Gln	gat Asp	att Ile	caa Gln 605	ttg Leu	ggt Gly	gct Ala	1824
aat Asn	ata Ile 610	acg Thr	ttg Leu	aat Asn	gtg Val	gaa Glu 615	gaa Glu	aac Asn	ttt Phe	gtt Val	aat Asn 620	cgt Arg	gca Ala	gga Gly	aca Thr	1872
ctg Leu 625	gca Ala	act Thr	ggt Gly	aaa Lys	aca Thr 630	ctg Leu	aca Thr	att Ile	aat Asn	acc Thr 635	gaa Glu	agt Ser	ggc Gly	agt Ser	att Ile 640	1920
tac Tyr	aat Asn	ctt Leu	ggt Gly	ggg Gly 645	aca Thr	tta Leu	ggt Gly	gct Ala	gga Gly 650	aaa Lys	tca Ser	tta Leu	aaa Lys	ctg Leu 655	act Thr	1968
gct Ala	aaa Lys	tca Ser	acg Thr 660	gaa Glu	gaa Glu	ggt Gly	atg Met	gga Gly 665	aat Asn	att Ile	gtt Val	aac Asn	caa Gln 670	gaa Glu	aac Asn	2016
ggt Gly	tta Leu	ttc Phe 675	cat His	aca Thr	ctc Leu	ggt Gly	aat Asn 680	atg Met	atg Met	tta Leu	gaa Glu	gca Ala 685	gag Glu	cgt Arg	tct Ser	2064
gtt Val	tat Tyr 690	aat Asn	att Ile	ggc Gly	gat Asp	att Ile 695	tat Tyr	gcg Ala	agt Ser	aaa Lys	aaa Lys 700	tta Leu	aca Thr	gtt Val	cat His	2112
act Thr 705	cat His	aat Asn	ttg Leu	att Ile	aat Asn 710	gat Asp	gtg Val	cgt Arg	tta Leu	tct Ser 715	ggc Gly	aat Asn	gtg Val	agt Ser	tat Tyr 720	2160
aag Lys	cct Pro	atc Ile	ggt Gly	tca Ser 725	agt Ser	cgt Arg	gat Asp	tat Tyr	gat Asp 730	atc Ile	agt Ser	cgt Arg	gtt Val	gcg Ala 735	gta Val	2208
cat His	ggt Gly	tgg Trp	cac His 740	aat Asn	aat Asn	gtt Val	tat Tyr	aag Lys 745	ctc Leu	aac Asn	tta Leu	aat Asn	ctg Leu 750	caa Gln	gaa Glu	2256
caa Gln	gat Asp	aaa Lys 755	acc Thr	gat Asp	att Ile	aaa Lys	gtt Val 760	gtg Val	aaa Lys	atg Met	ggg Gly	gct Ala 765	atc Ile	cgt Arg	tct Ser	2304
gat Asp	ggt Gly 770	gat Asp	ttt Phe	gac Asp	ttt Phe	aag Lys 775	gga Gly	ata Ile	aag Lys	gcg Ala	aca Thr 780	tca Ser	tca Ser	gaa Glu	tca Ser	2352

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	gcg Ala																2448
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	caa Gln																2544
tcg Ser	cgt Arg 850	gaa Glu	ttt Phe	aac Asn	aat Asn	tta Leu 855	gag Glu	tct Ser	ttc Phe	ctc Leu	gat Asp 860	gcc Ala	ttg Leu	ttt Phe	ggo	:	2592
tca Ser 865	aca Thr	aca Thr	atc Ile	tta Leu	aaa Lys 870	tca Ser	agt Ser	ttc Phe	tat Tyr	agt Ser 875	acg Thr	gaa Glu	aat Asn	ttt Phe	agt Ser 880	•	2640
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gag Glu	atg Met	cga Arg 915	aac Asn	aaa Lys	tgg Trp	aaa Lys	agc Ser 920	ttt Phe	aaa Lys	gaa Glu	aat Asn	cca Pro 925	aca Thr	gat Asp	ttc Phe	! .	2784
att Ile	tat Tyr 930	tac Tyr	cca Pro	tca Ser	gaa Glu	aaa Lys 935	gca Ala	aaa Lys	atc Ile	cta Leu	gcg Ala 940	gga Gly	aaa Lys	cta Leu	gaa Glu	. :	2832
ggt Gly 945	aag Lys	ctt Leu	aca Thr	acg Thr	cta Leu 950	caa Gln	aat Asn	ggt Gly	gaa Glu	tat Tyr 955	gcc Ala	gaa Glu	cgt Arg	ggt Gly	aag Lys 960		2880
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ggg Gly	gta Val	gat Asp 995	tta Leu	tcc Ser	tcg Ser	atc Ile	gcc Ala 1000	Glu	ctc Leu	tta Leu	gaa Glu	atg Met 100	Pr	ca aa co As	c t n L	ta eu	3024
ttt Phe	att Ile 1010	Asp	aat Asn	agt Ser	ato Ile	caa Gln 101	Le	a ga u Gl	a aa u Ly	g aa s Ly	s Ly	g t s I 20	tg t	ct c Ser P	ro	att Ile	3072
gag Glu 1025	Asp		gat Asp		ga G1 103	u Pr	a cg o Ar	t aa g Ly	a aa s As	n Le	g g u A 35	at a sp I	ta g le C	gaa g Slu G	lu	agc Ser 1040	3120

cat tct aat tca tcg His Ser Asn Ser Ser 1045	Asp Asp Val Leu Ser	atg aat gat gat gag tct 316 Met Asn Asp Asp Glu Ser 1055	58
	aag tgg agt atg ggc Lys Trp Ser Met Gly 1065	aat gat gag aaa gag atg 321 Asn Asp Glu Lys Glu Met 1070	16
ccc gat gat aag ctg	ggt ata agt cgt gat	gat cgt gga aat aaa cca 326	54
Pro Asp Asp Lys Leu	Gly Ile Ser Arg Asp	Asp Arg Gly Asn Lys Pro	
1075	1080	1085	
cct cgt act gat cct	aca gtt gat tat ctt	aac cct gat gaa ttc ttt 331	٠2
Pro Arg Thr Asp Pro	Thr Val Asp Tyr Leu	Asn Pro Asp Glu Phe Phe	
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Val Arg Leu Gly Glu	Arg Asp Arg Gln Asn	Arg Glu Lys Arg Glu Lys	
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Ile Glu Lys Ala Leu	Leu Gln Lys Ser Glu	Gln Gln Glu Lys Arg Val	
1185	1190	1195 1200	
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Glu Glu Arg Lys Gln	Glu Glu Lys Arg Gln	Ala Gln Asp Lys Ile Ala	
1205	1210	1215	
	gca aaa gaa atg caa Ala Lys Glu Met Gln 1225	cgg gta gaa gaa att cgc 3690 Arg Val Glu Glu Ile Arg 1230	6
cag aga gaa aaa caa	ctt gcg atc caa ctg	caa gaa gaa gag aag aaa 3744	4
Gln Arg Glu Lys Gln	Leu Ala Ile Gln Leu	Gln Glu Glu Lys Lys	
1235	1240	1245	
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1265	1270	1275 1280	
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Gln Gln Lys Ala Tyr	Glu Glu Met Ala Lys	Arg Glu Ala Glu Ala Ser	
1285	1290	1295	

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	caa 4128 Gln
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aaa acg aag gta aag ggc aaa gat gtg ttt gtt cca aag gtt tat t Lys Thr Lys Val Lys Gly Lys Asp Val Phe Val Pro Lys Val Tyr P	
	Phe 1440
gct tct gaa acg ctc gta gaa gcc caa aaa tta caa ggt tta ggc a	
gct tct gaa acg ctc gta gaa gcc caa aaa tta caa ggt tta ggc a Ala Ser Glu Thr Leu Val Glu Ala Gln Lys Leu Gln Gly Leu Gly T	1440 act 4368 Thr gtg 4416
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gct tct gaa acg ctc gta gaa gcc caa aaa tta caa ggt tta ggc a Ala Ser Glu Thr Leu 1445	1440 act 4368 Thr gtg 4416 Val aat 4464 Asn
gct tct gaa acg ctc Ala Ser Glu Thr Leu 1445 ggg act atc aga gtt ggt gaa gct aag att aaa gcc aaa gat gtg gGly Thr Ile Arg Val Gly Glu Ala Lys Ile Lys Ala Lys Asp Val Val 600 aat acc ggg aca tta gct ggg aga aaa ctc aat gtt gaa gcg agt at Asn Thr Gly Thr Leu Ala Gly Arg Lys Leu Asn Val Glu Ala Ser A 1485 aaa atc aaa aat caa ggg agt atc tta agt act caa gaa aca cgt t Lys Ile Lys Asn Gln Gly Ser Ile Leu Ser Thr Gln Glu Thr Arg L 1490 gtc ggg cgt aaa ggt att gaa ac gta tct cgt tca ttt gca aat gval Gly Arg Lys Leu Asn Val Glu Ala Ser Arg Ser Phe Ala Asn Asn Asn Arg Lys Gly Arg Lys Gly Ile Glu Asn Val Ser Arg Ser Phe Ala Asn Asn Arg Lys Gly Arg Lys Gly Ile Glu Asn Val Ser Arg Ser Phe Ala Asn Asn Asn Arg Ser Phe Ala Asn Asn Arg Lys Gly Ile Glu Asn Val Ser Arg Ser Phe Ala Asn Asn Arg Lys Gly Ile Glu Asn Val Ser Arg Ser Phe Ala Asn Arg Lys Gly Ile Glu Asn Val Ser Arg Ser Phe Ala Asn Arg Lys Clu A	1440 act 4368 Thr gtg 4416 Val aat 4464 Asn cta 4512 Leu gat 4560
gct tct gaa acg ctc Ala Ser Glu Thr Leu 1445 ggg act atc aga gtt ggt gaa gct aag att aaa gcc aaa gat gtg gg Gly Thr Ile Arg 1460 aat acc ggg aca tta gct ggg aga aaa ctc aat gtt gaa gcg agt a Asn Thr Gly 1475 aaa atc aaa aat caa ggg agt atc tta agt act caa gaa aca cgt t 1475 aaa atc aaa aat caa ggg agt atc tta agt act caa gaa aca cgt t 1475 aaa atc aaa aat caa ggg agt atc tta agt act caa gaa aca cgt t 1490 gtc ggg cgt aaa ggt att gaa aac gta tct cgt tca ttt gca aat g Val Gly Arg Lys Gly Ile Glu Asn Val Ser Arg Ser Phe Ala Asn A 1505 gaa tta gga gtc act gca caa cgc tca gaa atc aaa acg gaa ggt c gaa tta gga gtc act gca caa cgc tca gaa atc aaa acg gaa ggt c gaa tta gga gtc act gca caa cgc tca gaa atc aaa acg gaa ggt c gaa tta gga gtc act gca caa cgc tca gaa atc aaa acg gaa ggt c	1440 act 4368 Thr gtg 4416 Val aat 4464 Asn tta 4512 Leu gat 4560 Asp

Asp Ile Lys Ala Lys Thr Ser Phe Val Lys Thr Gly Asp Val Asn Leu 1555 1560 1565	4704
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<400> 103	
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Met Asn Lys Asn Arg Tyr Lys Leu Ile Phe Ser Gln Val Lys Gly Cys	
Met Asn Lys Asn Arg Tyr Lys Leu Ile Phe Ser Gln Val Lys Gly Cys 1 5 10 15 Leu Val Pro Val Ala Glu Cys Ile Asn Ser Ala Ile Ser Asn Gly Ser	
Met Asn Lys Asn Arg Tyr Lys Leu Ile Phe Ser Gln Val Lys Gly Cys 1 5 10 15 Leu Val Pro Val Ala Glu Cys Ile Asn Ser Ala Ile Ser Asn Gly Ser 20 25 30 Ser Asp Ser Thr Ser Thr Ser Glu Gln Val Glu Glu Glu Pro Phe Leu	
Met Asn Lys Asn Arg Tyr Lys Leu Ile Phe Ser Gln Val Lys Gly Cys 1 10 15 Leu Val Pro Val Ala Glu Cys Ile Asn Ser Ala Ile Ser Asn Gly Ser 20 25 25 25 Ala Ile Ser Asn Gly Ser 30 Ser Asp Ser Thr Ser Thr Ser Glu Gln Val Glu Glu Pro Phe Leu 35 40 40 45 Leu Glu Gln Tyr Ser Leu Ser Ser Val Ser Leu Leu Val Lys Ser Thr	
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Met Asn Lys Asn Arg Tyr Lys Leu Ile Phe Ser Gln Val Lys Gly Cys 15 Leu Val Pro Val Ala Glu Cys Ile Asn Ser Ala Ile Ser Asn Gly Ser 25 Ser Asp Ser Thr Ser Thr Ser Glu Gln Val Glu Glu Glu Pro Phe Leu 35 Leu Glu Gln Tyr Ser Leu Ser Ser Val Ser Leu Leu Val Lys Ser Thr 50 Phe Asn Pro Val Ser Tyr Ala Met Gln Leu Thr Trp Lys Gln Leu Ser 80 Ile Leu Phe Leu Thr Val Ile Ser Val Pro Val Leu Ala Glu Gly Lys 90 Gly Asp Glu Arg Asn Gln Leu Thr Val Ile Asp Asn Ser Asp His Ile	

Lys 145	Gly	Ile	Ser	Asp	Asn 150	Arg	Phe	Glu	Lys	Phe 155		Ile	Pro	Asn	Se:
Ala	Val	Phe	Asn	Asn 165	Asn	Gly	Thr	Glu	Ala 170		Ala	Arg	Ser	Thr 175	
Ile	Gly	Tyr	Ile 180	Pro	Gln	Asn	Gln	Asn 185		Arg	Gly	Gly	Lys 190	Glu	Ala
Asp	Val	Ile 195	Leu	Asn	Gln	Val	Thr 200	Gly	Pro	Gln	Glu	Ser 205	_	Ile	Va]
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Gln 225	Asn	Gly	Ile	Thr	Leu 230	Asn	Gly	Val	Arg	Thr 235	Ile	Asn	Ser	Asp	Arg 240
Phe	Val	Ala	Thr	Thr 245	Ser	Glu	Leu	Ile	Asp 250	Pro	Asn	Gln	Met	Met 255	Let
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Asp	Gly	Leu 275	Lys	Tyr	Leu	Asp	Ile 280	Ile	Ala	Lys	Lys	Ile 285	Glu	Gln	Lys
Gln	Ser 290	Ile,	Thr	Ser	Gly	Asp 295	Asn	Ser	Glu	Ala	Lys 300	Thr	Asp	Val	Thr
Leu 305	Ile	Ala	Gly	Ser	Ser 310	Glu	Tyr	Asp	Leu	Ser 315	Lys	His	Glu	Leu	Lys 320
Lys	Thr	Ser	Gly	Glu 325	Asn	Val	Ser	Asn	Asp 330	Val	Ile	Ala	Ile	Thr 335	Gly
Ser	Ser	Thr	Gly 340	Ala	Met	His	Gly	Lys 345	Asn	Ile	Lys	Leu	Ile 350	Val	Thr
Asp	Lys	Gly 355	Ala	Gly	Val	Lys	His 360	Asp	Gly	Ile	Ile	Leu 365	Ser	Glu	Asn
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Ala	Leu 450	Ser	Ile	Glu	Gln	Asn 455	Ala	Lys	Leu	Val	Ala 460	Lys	Lys	Ile	Asp
Val 465	Ala	Thr	Glu	Thr	Leu 470	Thr	Asn	Ala	Gly	Arg 475	Ile	Tyr	Gly	Arg	Glu 480

Val Lys Leu Asp Thr Asn Asn Leu Ile Asn Asp Lys Glu Ile Tyr Ala Glu Arg Lys Leu Ser Ile Leu Thr Lys Gly Lys Asp Leu Glu Ile Ile 505 Gln Asp Arg Tyr Leu Ser Pro Leu Met Arg Val Lys Ser Ser Val Arg Phe Leu Gly Ser Pro Phe Phe Ser Ile Ser Pro Ser Met Leu Ala Ser 535 Leu Ser Ala Gln Phe Lys Pro Gly Phe Val Asn Lys Gly Leu Ile Glu Ser Ala Gly Ser Ala Glu Leu Thr Phe Lys Glu Lys Thr Ser Phe Leu Thr Glu Gly Asn Asn Phe Ile Arg Ala Lys Asp Ala Leu Thr Ile Asn Ala Gln Asn Ile Glu Ile Asp Lys Asn Gln Asp Ile Gln Leu Gly Ala Asn Ile Thr Leu Asn Val Glu Glu Asn Phe Val Asn Arg Ala Gly Thr Leu Ala Thr Gly Lys Thr Leu Thr Ile Asn Thr Glu Ser Gly Ser Ile Tyr Asn Leu Gly Gly Thr Leu Gly Ala Gly Lys Ser Leu Lys Leu Thr 645 650 Ala Lys Ser Thr Glu Glu Gly Met Gly Asn Ile Val Asn Gln Glu Asn 665 Gly Leu Phe His Thr Leu Gly Asn Met Met Leu Glu Ala Glu Arg Ser 680 Val Tyr Asn Ile Gly Asp Ile Tyr Ala Ser Lys Lys Leu Thr Val His 695 Thr His Asn Leu Ile Asn Asp Val Arg Leu Ser Gly Asn Val Ser Tyr Lys Pro Ile Gly Ser Ser Arg Asp Tyr Asp Ile Ser Arg Val Ala Val His Gly Trp His Asn Asn Val Tyr Lys Leu Asn Leu Asn Leu Gln Glu 745 Gln Asp Lys Thr Asp Ile Lys Val Val Lys Met Gly Ala Ile Arg Ser Asp Gly Asp Phe Asp Phe Lys Gly Ile Lys Ala Thr Ser Ser Glu Ser Lys Pro Gln Leu Ile Asn His Gly Leu Ile Asn Val Lys Gly Thr Phe Asn Ala Glu Ala Asp Gln Val Val Asn Gln Met Lys Ala Phe Asn Gln 805 810

Asn Ala Leu Ala Ser Val Phe Lys Asn Pro Ala Lys Ile Thr Met Tyr 820 825 830

- Tyr Gln Pro Leu Thr Arg Tyr Ile Trp Thr Pro Leu Ser Gly Asn Ala 835 840 845
- Ser Arg Glu Phe Asn Asn Leu Glu Ser Phe Leu Asp Ala Leu Phe Gly 850 855 860
- Ser Thr Thr Ile Leu Lys Ser Ser Phe Tyr Ser Thr Glu Asn Phe Ser 865 870 875 880
- Ala Tyr Gln Leu Leu Ser His Ile Gln His Ser Pro Met Tyr Gln Lys 885 890 895
- Ala Met Ala Gln Val Phe Gly Ala Glu Trp His Ser Lys Ser Tyr Asp 900 905 910
- Glu Met Arg Asn Lys Trp Lys Ser Phe Lys Glu Asn Pro Thr Asp Phe 915 920 925
- Ile Tyr Tyr Pro Ser Glu Lys Ala Lys Ile Leu Ala Gly Lys Leu Glu 930 935 940
- Gly Lys Leu Thr Thr Leu Gln Asn Gly Glu Tyr Ala Glu Arg Gly Lys 945 950 955 960
- Phe Asp Glu Ser Ile Gln Ile Gly Lys His Gln Leu Ser Leu Pro Ser 965 970 975
- Val Glu Leu Lys Ala Glu Phe Ser Asp Lys Glu Arg Leu Glu Glu Asp 980 985 990
- Gly Val Asp Leu Ser Ser Ile Ala Glu Leu Leu Glu Met Pro Asn Leu 995 1000 1005
- Phe Ile Asp Asn Ser Ile Gln Leu Glu Lys Lys Leu Ser Pro Ile 1010 1015 1020
- Glu Asp Leu Asp Glu Glu Pro Arg Lys Asn Leu Asp Ile Glu Glu Ser 1025 1030 1035 1040
- His Ser Asn Ser Ser Asp Asp Val Leu Ser Met Asn Asp Asp Glu Ser 1045 1050 1055
- Asp Thr Asp Asp Ser Lys Trp Ser Met Gly Asn Asp Glu Lys Glu Met 1060 1065 1070
- Pro Asp Asp Lys Leu Gly Ile Ser Arg Asp Asp Arg Gly Asn Lys Pro 1075 1080 1085
- Pro Arg Thr Asp Pro Thr Val Asp Tyr Leu Asn Pro Asp Glu Phe Phe 1090 1095 1100
- Glu Asn Gly Tyr Leu Leu Asn Glu Leu Leu Gln Glu Leu Gly Glu Glu 1105 1110 1115
- Pro Leu Leu Lys Glu Gly Glu Asp His Phe Lys Arg Ser Thr Asn Leu 1125 1130 1135
- Val Arg Leu Gly Glu Arg Asp Arg Gln Asn Arg Glu Lys 1140 1145 1150

Glu Gly Tyr Phe Asp Leu Pro Gly Thr Leu Asp Met Lys Leu Gln Glu 1155 1160 1165

- Leu Phe Glu Lys Arg Lys Gln Lys His Glu Ala Glu Gln Lys Ala Arg 1170 1175 1180
- Ile Glu Lys Ala Leu Leu Gln Lys Ser Glu Gln Glu Lys Arg Val 1185 1190 1195 1200
- Glu Glu Arg Lys Gln Glu Glu Lys Arg Gln Ala Gln Asp Lys Ile Ala 1205 1210 1215
- Lys Gln Val Glu Ile Ala Lys Glu Met Gln Arg Val Glu Glu Ile Arg 1220 1225 1230
- Gln Arg Glu Lys Gln Leu Ala Ile Gln Leu Gln Glu Glu Glu Lys Lys 1235 1240 1245
- Gln Glu Glu Lys His Leu Ser Glu Glu Lys Lys Gln Ala Glu Gln 1250 1255 1260
- Lys Gln Lys Ala Glu Glu Lys Val Ala Gln Glu Arg Leu Asp Ile Glu 1265 1270 1275 1280
- Gln Gln Lys Ala Tyr Glu Glu Met Ala Lys Arg Glu Ala Glu Ala Ser 1285 1290 1295
- Lys Asn Val Leu Lys Ala Ile Asp Glu Glu Arg Pro Lys Val Glu
 1300 1305 1310
- Thr Asp Pro Leu Phe Arg Thr Lys Leu Lys Tyr Ile Asn Gln Asp Asp 1315 1320 1325
- Tyr Ala Gly Ala Asn Tyr Phe Phe Asn Lys Val Gly Leu Asn Thr Lys 1330 1335 1340
- Gly His Gln Lys Val Asn Val Leu Gly Asp Asn Tyr Phe Asp His Gln 1345 1350 1355 1360
- Val Ile Thr Arg Ser Ile Glu Lys Lys Val Asp Asn His Leu Asn Gln 1365 1370 1375
- Lys Tyr Asn Leu Ser Asp Val Glu Leu Val Lys Gln Leu Met Asp Asn 1380 1385 1390
- Ser Thr Thr Gln Ala Gln Glu Leu Asp Leu Lys Leu Gly Ala Ala Leu 1395 1400 1405
- Thr Lys Glu Gln Gln Ala Asn Leu Thr Gln Asp Ile Val Trp Tyr Val 1410 1420
- Lys Thr Lys Val Lys Gly Lys Asp Val Phe Val Pro Lys Val Tyr Phe 1425 1430 1435 1440
- Ala Ser Glu Thr Leu Val Glu Ala Gln Lys Leu Gln Gly Leu Gly Thr 1445 1450 1455
- Gly Thr Ile Arg Val Gly Glu Ala Lys Ile Lys Ala Lys Asp Val Val 1460 1465 1470
- Asn Thr Gly Thr Leu Ala Gly Arg Lys Leu Asn Val Glu Ala Ser Asn 1475 1480 1485

Lys Ile Lys Asn Gln Gly Ser Ile Leu Ser Thr Gln Glu Thr Arg Leu
1490 1495 1500

Val Gly Arg Lys Gly Ile Glu Asn Val Ser Arg Ser Phe Ala Asn Asp 1505 1510 1515 1520

Glu Leu Gly Val Thr Ala Gln Arg Ser Glu Ile Lys Thr Glu Gly His 1525 1530 1535

Leu His Leu Glu Thr Asp Lys Asp Ser Thr Ile Asp Val Gln Ala Ser 1540 1545 1550

Asp Ile Lys Ala Lys Thr Ser Phe Val Lys Thr Gly Asp Val Asn Leu 1555 1560 1565

Lys Asn Thr Tyr Asn Thr Lys His Ala Tyr Arg Glu Lys Phe Ser Pro 1570 1575 1580

Ser Ala Leu Gln Val Ala Glu Leu Asp Val Ala Gly Leu Lys Val Pro 1585 1590 1595 1600

Leu Leu Gly Val Ser Val Ser Ile Gln Phe Ile Gln Ser Ile Leu Val 1605 1610 1615

Arg Gln Leu Gln Glu Gly Ser Ile Phe Glu Val Gly His Leu His Xaa 1620 1635

Ala Val Asp Arg Arg Cys Glu Pro Ser Gly Glu 1635 1640

<210> 104

<211> 2009

<212> DNA

<213> Pasteurella multocida

<220>

<223> hmbR

<220>

<221> CDS

<222> (1)..(2007)

<400> 104

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ccg cag gct gaa tcg act ata tct act tcc gca cgt tat tcg act gaa 96
Pro Gln Ala Glu Ser Thr Ile Ser Thr Ser Ala Arg Tyr Ser Thr Glu
20 25 30

cgt cat aat ggt aat att aat aat att gaa tac gaa aat gtt agt tcg 144
Arg His Asn Gly Asn Ile Asn Asn Ile Glu Tyr Glu Asn Val Ser Ser

40
45

ttg aaa gtt caa aaa ggg gca gct tct gta atg tat ggt agc ggt gcg 192 Leu Lys Val Gln Lys Gly Ala Ala Ser Val Met Tyr Gly Ser Gly Ala 50 55 60

tta ggt gga acc gtg gag ttt acc aca aaa gat att gag gac ttt gtc 240 Leu Gly Gly Thr Val Glu Phe Thr Thr Lys Asp Ile Glu Asp Phe Val 65 70 75 80

gaa Glu	cct Pro	ggt Gly	cgc Arg	cat His 85	ttg Leu	ggc Gly	ttt Phe	ttg Leu	tct Ser 90	aaa Lys	acc Thr	ggc	tat Tyr	act Thr 95	tca Ser	288
aaa Lys	aac Asn	aga Arg	gaa Glu 100	tat Tyr	cgt Arg	caa Gln	gtc Val	atc Ile 105	gga Gly	gtt Val	gga Gly	gly	aaa Lys 110	999 Gly	gaa Glu	336
cac His	ttt Phe	ttt Phe 115	ggt Gly	ttt Phe	gta Val	caa Gln	tta Leu 120	acc Thr	aaa Lys	cgt Arg	tgg Trp	999 Gly 125	cat His	gaa Glu	aca Thr	384
												cat His				432
ccc Pro 145	aat Asn	ccg Pro	ctc Leu	aac Asn	tac Tyr 150	tat Tyr	act Thr	aca Thr	tca Ser	tgg Trp 155	tta Leu	acg Thr	aaa Lys	gtc Val	ggt Gly 160	480
tac Tyr	gat Asp	att Ile	aat Asn	aac Asn 165	act Thr	cat His	cgt Arg	ttt Phe	aca Thr 170	ctg Leu	ttt Phe	tta Leu	gaa Glu	gat Asp 175	cgc Arg	528
cgt Arg	gaa Glu	aag Lys	aag Lys 180	ctt Leu	acc Thr	gaa Glu	gaa Glu	aaa Lys 185	aca Thr	tta Leu	ggg Gly	ctt Leu	agt Ser 190	gat Asp	gca Ala	576
gtg Val	cgt Arg	ttt Phe 195	gct Ala	aat Asn	gat Asp	caa Gln	acc Thr 200	cct Pro	tat Tyr	ctc Leu	cgt Arg	tat Tyr 205	ggt Gly	att Ile	gaa Glu	624
tat Tyr	cga Arg 210	tat Tyr	aac Asn	ggc Gly	ttg Leu	tct Ser 215	tgg Trp	ttg Leu	gaa Glu	acg Thr	gta Val 220	aag Lys	ctt Leu	ttt Phe	ttg Leu	672
gca Ala 225	aag Lys	cag Gln	aaa Lys	atc Ile	gaa Glu 230	caa Gln	cgt Arg	tct Ser	gct Ala	ctc Leu 235	caa Gln	gag Glu	ttt Phe	gat Asp	att Ile 240	720
aat Asn	aat Asn	agg Arg	aat Asn	aaa Lys 245	ttg Leu	gat Asp	tcg Ser	act Thr	atg Met 250	tcg Ser	ttt Phe	gta Val	tat Tyr	tta Leu 255	caa Gln	768
aga Arg	cag Gln	aat Asn	ata Ile 260	gct Ala	cgg Arg	gga Gly	gaa Glu	ttt Phe 265	tca Ser	acg Thr	agt Ser	cct Pro	tta Leu 270	tat Tyr	tgg Trp	816
ggg Gly	ccg Pro	agt Ser 275	cgc Arg	cat His	cgt Arg	tta Leu	tct Ser 280	gcg Ala	aaa Lys	ttc Phe	gaa Glu	ttt Phe 285	cgt Arg	gat Asp	aag Lys	864
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aat Asn 305	aga Arg	ttc Phe	aga Arg	caa Gln	caa Gln 310	ggt Gly	cga Arg	aat Asn	aac Asn	tat Tyr 315	aca Thr	gaa Glu	gtg Val	ttt Phe	ccc Pro 320	960
gtt Val	aaa Lys	tcc Ser	cga Arg	gag Glu 325	ttt Phe	tct Ser	ttt Phe	tct Ser	ctt Leu 330	atg Met	gac Asp	gac Asp	att Ile	aag Lys 335	att Ile	1008

ggc Gly	gaa Glu	ttg Leu	cta Leu 340	cat His	ctc Leu	gga Gly	ttg Leu	ggc Gly 345	ggt Gly	cgg Arg	tgg Trp	gat Asp	cac His 350	tat Tyr	aac Asn	1056
					aat Asn											1104
					aca Thr										tta Leu	1152
gag Glu 385	tat Tyr	caa Gln	tta Leu	cat His	cca Pro 390	tca Ser	cat His	caa Gln	att Ile	gca Ala 395	tac Tyr	cgt Arg	tta Leu	agt Ser	acc Thr 400	1200
ggt Gly	ttt Phe	agg Arg	gtt Val	ccc Pro 405	cgt Arg	gtt Val	gaa Glu	gat Asp	ctt Leu 410	tat Tyr	ttt Phe	gaa Glu	gac Asp	cga Arg 415	gga Gly	1248
aaa Lys	agt Ser	tct Ser	tca Ser 420	caa Gln	ttt Phe	ctt Leu	cct Pro	aac Asn 425	ccc Pro	gat Asp	cta Leu	caa Gln	ccg Pro 430	gaa Glu	act Thr	1296
gca Ala	ctg Leu	aat Asn 435	cat His	gaa Glu	ata Ile	agt Ser	tac Tyr 440	cgt Arg	ttc Phe	caa Gln	aat Asn	caa Gln 445	tat Tyr	gcc Ala	cat His	1344
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cgt Arg 465	gag Glu	atg Met	acc Thr	tgt Cys	gat Asp 470	aaa Lys	att Ile	cca Pro	tat Tyr	gag Glu 475	tat Tyr	aat Asn	agg Arg	act Thr	tat Tyr 480	1440
gga Gly	tat Tyr	tgc Cys	acg Thr	cat His 485	aat Asn	act Thr	tat Tyr	gta Val	atg Met 490	ttt Phe	gtt Val	aat Asn	gaa Glu	cct Pro 495	gaa Glu	1488
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aaa Lys	ggt Gly 530	caa Gln	aat Asn	cat His	gac Asp	ggc Gly 535	gat Asp	ccg Pro	tta Leu	aaa Lys	tct Ser 540	att Ile	caa Gln	cca Pro	tgg Trp	1632
aca Thr 545	gtg Val	gta Val	acc Thr	ggt Gly	att Ile 550	gat Asp	tac Tyr	gaa Glu	act Thr	gaa Glu 555	999 Gly	tgg Trp	agc Ser	gtg Val	agt Ser 560	1680
ttg Leu	agc Ser	G1y 999	cgt Arg	tat Tyr 565	agt Ser	gcg Ala	gct Ala	aaa Lys	aaa Lys 570	gcc Ala	aaa Lys	gat Asp	gcg Ala	ata Ile 575	gaa Glu	1728
acg																

agt Ser	cca Pro	ser 595	tac Tyr	ttt Phe	gtt Val	gtt Val	gat Asp 600	Phe	acg Thr	ggg Gly	caa Gln	gtt Val 605	aac Asn	ctc Leu	agt Ser	1824
aaa Lys	aat Asn 610	gtc Val	att Ile	ttg Leu	aat Asn	atg Met 615	gjå aaa	gta Val	ttt Phe	aac Asn	ttg Leu 620	ttc Phe	aat Asn	cgt Arg	gat Asp	1872
	atg Met															1920
tcc Ser	cgt Arg	tct Ser	gtc Val	cgt Arg 645	gct Ala	aac Asn	agc Ser	cca Pro	ggc Gly 650	att Ile	aat Asn	cgg Arg	ttt Phe	acc Thr 655	gca Ala	1968
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<210> 105

<211> 669

<212> PRT

<213> Pasteurella multocida

<400> 105

Ile Arg Gly Val Asp Lys Asp Arg Val Ala Val Ile Val Asp Gly Ile

1 5 10 15

Pro Gln Ala Glu Ser Thr Ile Ser Thr Ser Ala Arg Tyr Ser Thr Glu 20 25 30

Arg His Asn Gly Asn Ile Asn Asn Ile Glu Tyr Glu Asn Val Ser Ser

Leu Lys Val Gln Lys Gly Ala Ala Ser Val Met Tyr Gly Ser Gly Ala
50 60

Leu Gly Gly Thr Val Glu Phe Thr Thr Lys Asp Ile Glu Asp Phe Val 65 70 75 80

Glu Pro Gly Arg His Leu Gly Phe Leu Ser Lys Thr Gly Tyr Thr Ser 85 90 95

Lys Asn Arg Glu Tyr Arg Gln Val Ile Gly Val Gly Gly Lys Gly Glu 100 105 110

His Phe Phe Gly Phe Val Gln Leu Thr Lys Arg Trp Gly His Glu Thr 115 120 125

Ile Asn Asn Gly Lys Gly Thr Asp Ile Leu Gly Glu His Arg Gly Lys 130 135 140

Pro Asn Pro Leu Asn Tyr Tyr Thr Thr Ser Trp Leu Thr Lys Val Gly 145 150 155

Tyr Asp Ile Asn Asn Thr His Arg Phe Thr Leu Phe Leu Glu Asp Arg 165 170 175

Arg Glu Lys Lys Leu Thr Glu Glu Lys Thr Leu Gly Leu Ser Asp Ala 180 185 190

Val Arg Phe Ala Asn Asp Gln Thr Pro Tyr Leu Arg Tyr Gly Ile Glu Tyr Arg Tyr Asn Gly Leu Ser Trp Leu Glu Thr Val Lys Leu Phe Leu 215 Ala Lys Gln Lys Ile Glu Gln Arg Ser Ala Leu Gln Glu Phe Asp Ile 230 Asn Asn Arg Asn Lys Leu Asp Ser Thr Met Ser Phe Val Tyr Leu Gln 250 Arg Gln Asn Ile Ala Arg Gly Glu Phe Ser Thr Ser Pro Leu Tyr Trp Gly Pro Ser Arg His Arg Leu Ser Ala Lys Phe Glu Phe Arg Asp Lys 280 Phe Leu Glu Asn Met Asn Lys His Phe Thr Phe Arg Pro Trp Gln Ile 295 Asn Arg Phe Arg Gln Gln Gly Arg Asn Asn Tyr Thr Glu Val Phe Pro Val Lys Ser Arg Glu Phe Ser Phe Ser Leu Met Asp Asp Ile Lys Ile Gly Glu Leu Leu His Leu Gly Leu Gly Gly Arg Trp Asp His Tyr Asn Tyr Lys Pro Leu Leu Asn Ser Gln His Asn Ile Asn Arg Thr Gln Arg 360 Leu Pro Tyr Pro Lys Thr Ser Ser Lys Phe Ser Tyr Gln Leu Ser Leu 375 Glu Tyr Gln Leu His Pro Ser His Gln Ile Ala Tyr Arg Leu Ser Thr 390 395 Gly Phe Arg Val Pro Arg Val Glu Asp Leu Tyr Phe Glu Asp Arg Gly 410 Lys Ser Ser Ser Gln Phe Leu Pro Asn Pro Asp Leu Gln Pro Glu Thr 425 Ala Leu Asn His Glu Ile Ser Tyr Arg Phe Gln Asn Gln Tyr Ala His Phe Ser Val Gly Leu Phe Arg Thr Arg Tyr His Asn Phe Ile Gln Glu Arg Glu Met Thr Cys Asp Lys Ile Pro Tyr Glu Tyr Asn Arg Thr Tyr Gly Tyr Cys Thr His Asn Thr Tyr Val Met Phe Val Asn Glu Pro Glu Ala Val Ile Lys Gly Val Glu Val Ser Gly Ala Leu Asn Gly Ser Ala 505 Phe Gly Leu Ser Asp Gly Leu Thr Phe Arg Leu Lys Gly Ser Tyr Ser

Lys Gly Gln Asn His Asp Gly Asp Pro Leu Lys Ser Ile Gln Pro Trp 535 Thr Val Val Thr Gly Ile Asp Tyr Glu Thr Glu Gly Trp Ser Val Ser Leu Ser Gly Arg Tyr Ser Ala Ala Lys Lys Ala Lys Asp Ala Ile Glu Thr Glu Tyr Thr His Asp Lys Lys Val Val Lys Gln Trp Pro His Leu Ser Pro Ser Tyr Phe Val Val Asp Phe Thr Gly Gln Val Asn Leu Ser Lys Asn Val Ile Leu Asn Met Gly Val Phe Asn Leu Phe Asn Arg Asp Tyr Met Thr Trp Asp Ser Ala Tyr Asn Leu Phe Thr Arg Gly Tyr Thr Ser Arg Ser Val Arg Ala Asn Ser Pro Gly Ile Asn Arg Phe Thr Ala 645 Pro Lys Arg Asn Phe Ala Ala Ser Val Glu Ile Arg Phe <210> 106 <211> 908 <212> DNA <213> Pasteurella multocida <220> <223> lgtC <220> <221> CDS <222> (1)..(906) <400> 106 atg aat att tta ttt gtt tct gat gat gtt tat gct aaa cat ctg gtg Met Asn Ile Leu Phe Val Ser Asp Asp Val Tyr Ala Lys His Leu Val gtt gcg att aaa agc att ata aat cat aat gaa aaa ggt att tca ttt 96 Val Ala Ile Lys Ser Ile Ile Asn His Asn Glu Lys Gly Ile Ser Phe 20 25 tat att ttt gat ttg ggt ata aag gat gaa aat aag aga aat att aat Tyr Ile Phe Asp Leu Gly Ile Lys Asp Glu Asn Lys Arg Asn Ile Asn gat att gtt tot tot tat gga agt gaa gtc aac ttt att gct gtg aat Asp Ile Val Ser Ser Tyr Gly Ser Glu Val Asn Phe Ile Ala Val Asn 55 gag aaa gaa ttt gag agt ttt cct gtt caa att agt tat att tct tta Glu Lys Glu Phe Glu Ser Phe Pro Val Gln Ile Ser Tyr Ile Ser Leu 70 65

ac.	363	tat	a ca	200	a+ a		~~~	~~~	~-~			222	an t		.	200
			gca Ala													288
aat Asn	aaa Lys	att Ile	att Ile 100	tat Tyr	tta Leu	gat Asp	gtt Val	gat Asp 105	gtt Val	ttg Leu	gtt Val	ttt Phe	aac Asn 110	tca Ser	tta Leu	336
			tgg Trp													384
			ttc Phe													432
tca Ser 145	atg Met	tca Ser	gat Asp	aag Lys	gaa Glu 150	tat Tyr	tat Tyr	ttt Phe	aat Asn	gca Ala 155	gga Gly	gta Val	atg Met	cta Leu	ttt Phe 160	480
			gaa Glu													528
			atg Met 180													576
			ctt Leu													624
		_	cca Pro				_	_								672
	-	_	aac Asn					_			_	_		_	_	720
			tat Tyr													768
cat His	ttt Phe	aat Asn	gta Val 260	tat Tyr	ttc Phe	tat Tyr	cag Gln	aaa Lys 265	ata Ile	tta Leu	gca Ala	gaa Glu	ata Ile 270	acg Thr	aga Arg	816
			aaa Lys													864
			agg Arg											ta		908

<210> 107

<211> 302

<212> PRT

<213> Pasteurella multocida

<400> 107

Met Asn Ile Leu Phe Val Ser Asp Asp Val Tyr Ala Lys His Leu Val

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Val Ala Ile Lys Ser Ile Ile Asn His Asn Glu Lys Gly Ile Ser Phe
20 25 30

Tyr Ile Phe Asp Leu Gly Ile Lys Asp Glu Asn Lys Arg Asn Ile Asn 35 40 45

Asp Ile Val Ser Ser Tyr Gly Ser Glu Val Asn Phe Ile Ala Val Asn 50 55 60

Glu Lys Glu Phe Glu Ser Phe Pro Val Gln Ile Ser Tyr Ile Ser Leu 65 70 75 80

Ala Thr Tyr Ala Arg Leu Lys Ala Ala Glu Tyr Leu Pro Asp Asn Leu 85 90 95

Asn Lys Ile Ile Tyr Leu Asp Val Asp Val Leu Val Phe Asn Ser Leu 100 105 110

Glu Met Leu Trp Asn Val Asp Val Asn Asn Phe Leu Thr Ala Ala Cys 115 120 125

Tyr Asp Ser Phe Ile Glu Asn Glu Lys Ser Glu His Lys Lys Ser Ile 130 135 140

Ser Met Ser Asp Lys Glu Tyr Tyr Phe Asn Ala Gly Val Met Leu Phe 145 150 155 160

Asn Leu Asp Glu Trp Arg Lys Met Asp Val Phe Ser Arg Ala Leu Asp 165 170 175

Leu Leu Ala Met Tyr Pro Asn Gln Met Ile Tyr Gln Asp Gln Asp Ile 180 185 190

Leu Asn Ile Leu Phe Arg Asn Lys Val Cys Tyr Leu Asp Cys Arg Phe 195 200 205

Asn Phe Met Pro Asn Gln Leu Glu Arg Ile Lys Gln Tyr His Lys Gly 210 215 220

Lys Leu Ser Asn Leu His Ser Leu Glu Lys Thr Thr Met Pro Val Val 225 230 235 240

Ile Ser His Tyr Cys Gly Pro Glu Lys Ala Trp His Ala Asp Cys Lys 245 250 255

His Phe Asn Val Tyr Phe Tyr Gln Lys Ile Leu Ala Glu Ile Thr Arg 260 265 270

Gly Thr Asp Lys Glu Arg Val Leu Ser Ile Lys Thr Tyr Leu Lys Ala 275 280 285

Leu Ile Arg Arg Ile Arg Tyr Lys Phe Lys Tyr Gln Val Tyr 290 295 300

<210> 108

<211> 2054

<212> DNA

<213> Pasteurella multocida

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gaa Glu 225	gca Ala	tac Tyr	cgt Arg	atc Ile	act Thr 230	gaa Glu	aaa Lys	caa Gln	gca Ala	cgt Arg 235	tat Tyr	gaa Glu	caa Gln	att Ile	gat Asp 240	720
gcg Ala	att Ile	aaa Lys	gct Ala	gat Asp 245	gtg Val	att Ile	gca Ala	caa Gln	atc Ile 250	aca Thr	gct Ala	gaa Glu	gta Val	gca Ala 255	gaa Glu	768
													acc Thr 270			816
													cca Pro			864
													tgt Cys			912
gtt Val 305	tta Leu	cca Pro	cgt Arg	aca Thr	cac His 310	ggt Gly	tct Ser	gcg Ala	att Ile	ttc Phe 315	acc Thr	cgt Arg	ggt Gly	gaa Glu	aca Thr 320	960
cag Gln	gcg Ala	tta Leu	gct Ala	gtc Val 325	gcg Ala	aca Thr	tta Leu	ggt Gly	aca Thr 330	gaa Glu	cgt Arg	gat Asp	gca Ala	caa Gln 335	att Ile	1008
att Ile	gat Asp	gaa Glu	tta Leu 340	aca Thr	ggt Gly	gag Glu	cgt Arg	tca Ser 345	gat Asp	cac His	ttc Phe	tta Leu	ttc Phe 350	cac His	tac Tyr	1056
aac Asn	ttc Phe	ccg Pro 355	cca Pro	tat Tyr	tct Ser	gtg Val	ggt Gly 360	gaa Glu	acc Thr	ggt Gly	atg Met	att Ile 365	ggt Gly	tca Ser	cca Pro	1104
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gca Ala 385	gtg Val	atg Met	cca Pro	aca Thr	ctt Leu 390	gcc Ala	gag Glu	ttc Phe	ccg Pro	tat Tyr 395	gtg Val	gta Val	cgt Arg	gtt Val	gtc Val 400	1200
tct Ser	gaa Glu	atc Ile	aca Thr	gaa Glu 405	tca Ser	aat Asn	ggt Gly	tct Ser	tct Ser 410	tct Ser	atg Met	gca Ala	tcg Ser	gtt Val 415	tgt Cys	1248
													aaa Lys 430			1296
gtt Val	gca Ala	ggt Gly 435	att Ile	gca Ala	atg Met	ggc Gly	tta Leu 440	gtc Val	aaa Lys	gaa Glu	gac Asp	gaa Glu 445	aaa Lys	ttt Phe	gtg Val	1344
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ttc Phe 465	aaa Lys	gtc Val	gcg Ala	ggt Gly	aca Thr 470	cgt Arg	acg Thr	ggt Gly	gtg Val	acg Thr 475	gca Ala	tta Leu	caa Gln	atg Met	gat Asp 480	1440

		atc Ile														1488
		aaa Lys														1536
		gcg Ala 515														1584
		aaa Lys														1632
ggt Gly 545	gca Ala	acc Thr	att Ile	cgt Arg	gcc Ala 550	tta Leu	aca Thr	gaa Glu	gaa Glu	aca Thr 555	ggt Gly	acc Thr	tca Ser	att Ile	gat Asp 560	1680
atc Ile	gat Asp	gat Asp	gat Asp	ggt Gly 565	acg Thr	gtg Val	aag Lys	att Ile	gct Ala 570	gcg Ala	gtt Val	gat Asp	ggc Gly	aat Asn 575	tca Ser	1728
		gag Glu														1776
		gca Ala 595														1824
		gtt Val														1872
		gcg Ala														1920
		gaa Glu														1968
		tta Leu														2016
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<213> Pasteurella multocida

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35 40 45

- Arg Glu Gly Arg Pro Ser Glu Gly Glu Thr Leu Ile Ala Arg Leu Ile
 50 55 60
- Asp Arg Pro Ile Arg Pro Leu Phe Pro Glu Gly Phe Tyr Asn Glu Ile 65 70 75 80
- Gln Ile Val Ala Thr Val Val Ser Val Asn Pro Gln Ile Cys Pro Asp 85 90 95
- Leu Val Ala Met Ile Gly Ala Ser Ala Ala Leu Ser Leu Ser Gly Val 100 105 110
- Pro Phe Asn Gly Pro Ile Gly Ala Ala Arg Val Gly Phe Ile Asp Asp 115 120 125
- Gln Phe Val Leu Asn Pro Thr Met Asn Glu Gln Lys Gln Ser Arg Leu 130 135 140
- Asp Leu Val Val Ala Gly Thr Asp Lys Ala Val Leu Met Val Glu Ser 145 150 155 160
- Glu Ala Asp Val Leu Thr Glu Glu Gln Met Leu Ala Ala Val Val Phe
 165 170 175
- Gly His Gln Gln Gln Val Val Ile Asp Ala Ile Lys Glu Phe Thr 180 185 190
- Ala Glu Ala Gly Lys Pro Arg Trp Asp Trp Val Ala Pro Glu Pro Asn 195 200 205
- Thr Ala Leu Ile Glu Lys Val Lys Ala Ile Ala Glu Ala Arg Leu Gly . 210 $\,$ 220 $\,$
- Glu Ala Tyr Arg Ile Thr Glu Lys Gln Ala Arg Tyr Glu Gln Ile Asp 225 230 235
- Ala Ile Lys Ala Asp Val Ile Ala Gl
n Ile Thr Ala Glu Val Ala Glu 245 $$ 250 $$ 255
- Gly Glu Asp Ile Ser Glu Gly Lys Ile Val Asp Ile Phe Thr Ala Leu 260 265 270
- Glu Ser Gln Ile Val Arg Ser Arg Ile Ile Ala Gly Glu Pro Arg Ile 275 280 285
- Asp Gly Arg Thr Val Asp Thr Val Arg Ala Leu Asp Ile Cys Thr Gly 290 295 300
- Val Leu Pro Arg Thr His Gly Ser Ala Ile Phe Thr Arg Gly Glu Thr 305 310 315 320
- Gln Ala Leu Ala Val Ala Thr Leu Gly Thr Glu Arg Asp Ala Gln Ile 325 330 335
- Ile Asp Glu Leu Thr Gly Glu Arg Ser Asp His Phe Leu Phe His Tyr 340 345 350
- Asn Phe Pro Pro Tyr Ser Val Gly Glu Thr Gly Met Ile Gly Ser Pro

355 360 365

Lys Arg Arg Glu Ile Gly His Gly Arg Leu Ala Lys Arg Gly Val Ala 370 380

Ala Val Met Pro Thr Leu Ala Glu Phe Pro Tyr Val Val Arg Val Val 385 390 395 400

Ser Glu Ile Thr Glu Ser Asn Gly Ser Ser Ser Met Ala Ser Val Cys 405 410 415

Gly Ala Ser Leu Ala Leu Met Asp Ala Gly Val Pro Ile Lys Ala Ala 420 425 430

Val Ala Gly Ile Ala Met Gly Leu Val Lys Glu Asp Glu Lys Phe Val 435 440 445

Val Leu Ser Asp Ile Leu Gly Asp Glu Asp His Leu Gly Asp Met Asp 450 455 460

Phe Lys Val Ala Gly Thr Arg Thr Gly Val Thr Ala Leu Gln Met Asp 465 470 475 480

Ile Lys Ile Glu Gly Ile Thr Ala Glu Ile Met Gln Ile Ala Leu Asn 485 490 495

Gln Ala Lys Ser Ala Arg Leu His Ile Leu Gly Val Met Glu Gln Ala 500 505 510

Ile Pro Ala Pro Arg Ala Asp Ile Ser Asp Phe Ala Pro Arg Ile Tyr 515 520 525

Thr Met Lys Ile Asp Pro Lys Lys Ile Lys Asp Val Ile Gly Lys Gly 530 540

Gly Ala Thr Ile Arg Ala Leu Thr Glu Glu Thr Gly Thr Ser Ile Asp 545 550 555 560

Ile Asp Asp Asp Gly Thr Val Lys Ile Ala Ala Val Asp Gly Asn Ser
565 570 575

Ala Lys Glu Val Met Ala Arg Ile Glu Asp Ile Thr Ala Glu Val Glu 580 585 590

Ala Gly Ala Val Tyr Lys Gly Lys Val Thr Arg Leu Ala Asp Phe Gly 595 600

Ala Phe Val Ser Ile Val Gly Asn Lys Glu Gly Leu Val His Ile Ser 610 615 620

Gln Ile Ala Glu Glu Arg Val Glu Lys Val Ser Asp Tyr Leu Ala Val 625 630 635

Gly Gln Glu Val Thr Val Lys Val Val Glu Ile Asp Arg Gln Gly Arg
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Ile Tyr Asp Ala Leu Thr Leu Leu Gln His Arg Gly Gln Asp Ala Ala
                                 25
ggg att gta acc gta gat gat gaa aac cga ttc cgc ttg cgt aaa gcg
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Gly Ile Val Thr Val Asp Asp Glu Asn Arg Phe Arg Leu Arg Lys Ala
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aac ggg tta gtc agc gat gta ttt gaa caa gtt cat atg tta cgt tta
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Asn Gly Leu Val Ser Asp Val Phe Glu Gln Val His Met Leu Arg Leu
     50
caa ggc aat gct ggc att gga cat gtt cgt tat cct acg gct ggg agc
                                                                   240
Gln Gly Asn Ala Gly Ile Gly His Val Arg Tyr Pro Thr Ala Gly Ser
tca agt gtc tct gaa gcg caa cct ttt tat gta aat tcg cct tat ggc
                                                                   288
Ser Ser Val Ser Glu Ala Gln Pro Phe Tyr Val Asn Ser Pro Tyr Gly
                 85
tta acc tta gtg cat aat ggt aac ttg acc aat tca agt gaa tta aaa
Leu Thr Leu Val His Asn Gly Asn Leu Thr Asn Ser Ser Glu Leu Lys
gaa aag tta ttt cgt ctc gca cgt cgc cat gta aat acc aat tca gat
                                                                   384
Glu Lys Leu Phe Arg Leu Ala Arg Arg His Val Asn Thr Asn Ser Asp
                            120
tot gaa tta tta ctc aat atc tta gcc aat cac ctt gat cac ttc gaa
                                                                   432
Ser Glu Leu Leu Asn Ile Leu Ala Asn His Leu Asp His Phe Glu
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aaa tac caa tta gat ccg caa gat gta ttc agt gct gtc aaa caa acg
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Lys Tyr Gln Leu Asp Pro Gln Asp Val Phe Ser Ala Val Lys Gln Thr
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                                        155
cat cag gat att cgt ggt gct tat gct tgt atc gcc atg att att ggt
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His Gln Asp Ile Arg Gly Ala Tyr Ala Cys Ile Ala Met Ile Ile Gly
cat ggt atg gtc gcg ttt cgt gat ccg aac ggt atc cgt ccg tta gtg
                                                                  576
His Gly Met Val Ala Phe Arg Asp Pro Asn Gly Ile Arg Pro Leu Val
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tta ggg aaa cgc gag gaa aat ggc aaa aca gag tat atg ttt gcc tcc
Leu Gly Lys Arg Glu Glu Asn Gly Lys Thr Glu Tyr Met Phe Ala Ser
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gtc Val	tat Tyr	ttt Phe	gca Ala 260	cgt Arg	cca Pro	gac Asp	tct Ser	tgc Cys 265	atc Ile	gat Asp	ggg Gly	gtt Val	tct Ser 270	gtt Val	tat Tyr	816
gct Ala	gcc Ala	cgt Arg 275	gtt Val	cat His	atg Met	gga Gly	caa Gln 280	cgt Arg	tta Leu	ggt Gly	gaa Glu	aaa Lys 285	att Ile	gca Ala	cgg Arg	864
gaa Glu	tgg Trp 290	gcg Ala	gat Asp	gtg Val	gat Asp	gat Asp 295	att Ile	gat Asp	gtg Val	gtc Val	att Ile 300	cct Pro	gtg Val	cct Pro	gaa Glu	912
acc Thr 305	tct Ser	aac Asn	gat Asp	att Ile	gct Ala 310	tta Leu	cgt Arg	att Ile	gcg Ala	cgc Arg 315	gtg Val	tta Leu	aat Asn	aaa Lys	ccg Pro 320	960
tat Tyr	cgt Arg	caa Gln	ggt Gly	ttt Phe 325	gtg Val	aaa Lys	aat Asn	cgc Arg	tat Tyr 330	gta Val	gga Gly	cgt Arg	acg Thr	ttt Phe 335	att Ile	1008
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acc Thr	att Ile	gct Ala 355	tca Ser	gaa Glu	ttt Phe	aaa Lys	gat Asp 360	aag Lys	aat Asn	gtg Val	tta Leu	tta Leu 365	gtt Val	gac Asp	gac Asp	1104
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gcg Ala 385	gca Ala	ggt Gly	gcg Ala	aag Lys	aaa Lys 390	att Ile	tat Tyr	ttt Phe	gcc Ala	tct Ser 395	gct Ala	gca Ala	cca Pro	gaa Glu	att Ile 400	1200
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atc Ile	gct Ala	tat Tyr	ggt Gly 420	cgt Arg	gat Asp	gta Val	gat Asp	gaa Glu 425	att Ile	gct Ala	aac Asn	tta Leu	att Ile 430	ggt Gly	gtg Val	1296
gat Asp	aaa Lys	ttg Leu 435	att Ile	ttc Phe	caa Gln	gat Asp	ttg Leu 440	gat Asp	gcg Ala	tta Leu	act Thr	ggt Gly 445	tct Ser	gtg Val	caa Gln	1344
caa	gaa	aat	сса	agt	att	caa	gac	ttt	gat	tgt	tcg	gtg	ttt	aca	ggg	1392

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<213> Pasteurella multocida

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Gly Ile Val Thr Val Asp Asp Glu Asn Arg Phe Arg Leu Arg Lys Ala

Asn Gly Leu Val Ser Asp Val Phe Glu Gln Val His Met Leu Arg Leu

Gln Gly Asn Ala Gly Ile Gly His Val Arg Tyr Pro Thr Ala Gly Ser

Ser Ser Val Ser Glu Ala Gln Pro Phe Tyr Val Asn Ser Pro Tyr Gly

Leu Thr Leu Val His Asn Gly Asn Leu Thr Asn Ser Ser Glu Leu Lys

Glu Lys Leu Phe Arg Leu Ala Arg Arg His Val Asn Thr Asn Ser Asp 115 120

Ser Glu Leu Leu Asn Ile Leu Ala Asn His Leu Asp His Phe Glu 135

Lys Tyr Gln Leu Asp Pro Gln Asp Val Phe Ser Ala Val Lys Gln Thr 145

His Gln Asp Ile Arg Gly Ala Tyr Ala Cys Ile Ala Met Ile Ile Gly

His Gly Met Val Ala Phe Arg Asp Pro Asn Gly Ile Arg Pro Leu Val

Leu Gly Lys Arg Glu Glu Asn Gly Lys Thr Glu Tyr Met Phe Ala Ser

Glu Ser Ile Ala Leu Asp Thr Val Gly Phe Glu Phe Val Arg Asp Val 215

Gln Pro Gly Glu Ala Ile Tyr Val Thr Phe Glu Gly Glu Met Tyr Ala Gln Gln Cys Ala Asp Lys Pro Thr Leu Thr Pro Cys Ile Phe Glu Tyr Val Tyr Phe Ala Arg Pro Asp Ser Cys Ile Asp Gly Val Ser Val Tyr Ala Ala Arg Val His Met Gly Gln Arg Leu Gly Glu Lys Ile Ala Arg Glu Trp Ala Asp Val Asp Asp Ile Asp Val Val Ile Pro Val Pro Glu Thr Ser Asn Asp Ile Ala Leu Arg Ile Ala Arg Val Leu Asn Lys Pro 305 310 315 Tyr Arg Gln Gly Phe Val Lys Asn Arg Tyr Val Gly Arg Thr Phe Ile Met Pro Gly Gln Ala Leu Arg Val Ser Ser Val Arg Arg Lys Leu Asn 345 Thr Ile Ala Ser Glu Phe Lys Asp Lys Asn Val Leu Leu Val Asp Asp Ser Ile Val Arg Gly Thr Thr Ser Glu Gln Ile Val Glu Met Ala Arg Ala Ala Gly Ala Lys Lys Ile Tyr Phe Ala Ser Ala Ala Pro Glu Ile Arg Tyr Pro Asn Val Tyr Gly Ile Asp Met Pro Thr Lys Asn Glu Leu Ile Ala Tyr Gly Arg Asp Val Asp Glu Ile Ala Asn Leu Ile Gly Val 425 Asp Lys Leu Ile Phe Gln Asp Leu Asp Ala Leu Thr Gly Ser Val Gln Gln Glu Asn Pro Ser Ile Gln Asp Phe Asp Cys Ser Val Phe Thr Gly 455 Val Tyr Val Thr Gly Asp Ile Thr Pro Glu Tyr Leu Asp Asn Ile Ala Glu Gln Arg Asn Asp Ile Ala Lys Lys Lys Arg Glu Lys Asp Ala Thr Asn Leu Glu Met His Asn Glu Lys

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<213> Pasteurella multocida

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					aga Arg 230											720
					aaa Lys											768
					tta Leu											816
tgt Cys	gga Gly	ctt Leu 275	gaa Glu	cat His	ctc Leu	cat His	ttt Phe 280	cat His	gat Asp	acg Thr	aga Arg	agg Arg 285	gaa Glu	gcg Ala	ttg Leu	864
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<211> 329

<212> PRT

<213> Pasteurella multocida

<400> 113

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Lys Asn Gly Val Arg Lys Ser Ala Thr Phe Lys Thr Lys Ser Glu Ala 20 25 30

Asm Ala Trp Ala Val Asp Glu Glu Arg Lys Leu Ala Asp Leu Ala Lys 35 40 45

Gly Ile Ala Pro Asp Ile Ile Phe Arg Asp Val Ile Glu Arg Tyr Gln
50 55 60

Asn Glu Val Ser Ile Thr Lys Lys Gly Ala Arg Asn Glu Ile Ile Arg 65 70 75 80

Leu Asn Arg Phe Leu Arg Tyr Asp Ile Ser Asn Leu Tyr Ile Arg Asp 85 90 95

Leu Arg Lys Glu Asp Phe Glu Glu Trp Ile Arg Ile Arg Leu Thr Glu 100 105 110

Val Ser Asp Ala Ser Val Arg Glu Leu Val Thr Ile Ser Ser Val 115 120 125

Leu Thr Thr Ala Ile Asn Lys Trp Gly Tyr Ile Ser Arg His Pro Met 130 135 140

Thr Gly Ile Glu Lys Pro Lys Asn Ser Ala Glu Arg Lys Glu Arg Tyr

155

160

150

145

Ser Glu Gln Asp Ile Lys Thr Ile Leu Glu Thr Ala Arg Tyr Cys Glu 175 Asp Lys Leu Pro Ile Thr Leu Lys Gln Arg Val Ala Ile Ala Met Leu 185 Phe Ala Ile Glu Thr Ala Met Arg Ala Gly Glu Ile Ala Ser Ile Lys 200 Trp Asp Asn Val Phe Leu Glu Lys Arg Ile Val His Leu Pro Thr Thr Lys Asn Gly His Ser Arg Asp Val Pro Leu Ser Gln Arg Ala Val Ala 230 Leu Ile Leu Lys Met Lys Glu Val Glu Asn Gly Asp Leu Val Phe Gln Thr Thr Pro Glu Ser Leu Ser Thr Thr Phe Arg Val Leu Lys Lys Glu 265 Cys Gly Leu Glu His Leu His Phe His Asp Thr Arg Arg Glu Ala Leu Thr Arg Leu Ser Lys Lys Val Asp Val Met Thr Leu Ala Lys Ile Ser 290 295 Gly His Arg Asp Leu Arg Ile Leu Gln Asn Thr Tyr Tyr Ala Pro Asn Met Ser Glu Val Ala Asn Leu Leu Asp 325 <210> 114 <211> 1190 <212> DNA <213> Pasteurella multocida <220> <223> sopE <220> <221> CDS <222> (1)..(1188) <400> 114 atg tct gaa gaa tat cta cat ggt gtc aaa gtc aca gaa atc aat caa Met Ser Glu Glu Tyr Leu His Gly Val Lys Val Thr Glu Ile Asn Gln gca att cgc aca att caa agt cta tca acc gca gtc atc ggt att gtc Ala Ile Arg Thr Ile Gln Ser Leu Ser Thr Ala Val Ile Gly Ile Val 20 tgt act gca aat gac gca gac aat gaa aca ttc cca ctc aat gaa ccc Cys Thr Ala Asn Asp Ala Asp Asn Glu Thr Phe Pro Leu Asn Glu Pro 35 gtt ctc atc aca aac gtg gca gcg gca att ggc aag gct gga aaa caa Val Leu Ile Thr Asn Val Ala Ala Ile Gly Lys Ala Gly Lys Gln

55 60 ggc acg ctt tca cgt gcg ctt gac ggg att tct gat gta gtc aat tgc 240 Gly Thr Leu Ser Arg Ala Leu Asp Gly Ile Ser Asp Val Val Asn Cys aaa gtg att gtt gtg cga gtg caa gaa agt gcg caa gaa gac gaa qaa 288 Lys Val Ile Val Val Arg Val Gln Glu Ser Ala Gln Glu Asp Glu Glu 85 aca aaa gca agt gaa atg aac acg gca att att ggc aca atc aca gaa 336 Thr Lys Ala Ser Glu Met Asn Thr Ala Ile Ile Gly Thr Ile Thr Glu gaa ggg cag tac aca ggc ttg aag gcg tta ttg att gcg aaa aac aaa Glu Gly Gln Tyr Thr Gly Leu Lys Ala Leu Leu Ile Ala Lys Asn Lys 115 120 ttc ggt atc aaa cca cgt att tta tgt gtg cca aaa ttc gac aca aaa 432 Phe Gly Ile Lys Pro Arg Ile Leu Cys Val Pro Lys Phe Asp Thr Lys gaa gtc gcc aca gag ctt gca agt atc gcc gcc aaa ctc aac gca ttt 480 Glu Val Ala Thr Glu Leu Ala Ser Ile Ala Ala Lys Leu Asn Ala Phe 150 155 gct tac att tca tgt caa ggg tgt aaa acg aaa gaa caa gcg gtg caa 528 Ala Tyr Ile Ser Cys Gln Gly Cys Lys Thr Lys Glu Gln Ala Val Gln 165 tat aaa cgc aac ttc tca caa cgt gaa gtc atg ctg atc atg ggc gat 576 Tyr Lys Arg Asn Phe Ser Gln Arg Glu Val Met Leu Ile Met Gly Asp 185 ttt ctg tca ttt aat gtc aac aca tca aaa gtt gag att gac tat gcc 624 Phe Leu Ser Phe Asn Val Asn Thr Ser Lys Val Glu Ile Asp Tyr Ala 195 200 gtc act cgt gcg gcg gca atg cgt gca tat ctt gat aaa gaa cag ggc 672 Val Thr Arg Ala Ala Ala Met Arg Ala Tyr Leu Asp Lys Glu Gln Gly tgg cat acg tct att tca aat aaa ggc att aat ggc gtg agc ggt gtc 720 Trp His Thr Ser Ile Ser Asn Lys Gly Ile Asn Gly Val Ser Gly Val 230 aca caa cca ctc tat ttt gac att aac gac agc tcg act gat gtg aac 768 Thr Gln Pro Leu Tyr Phe Asp Ile Asn Asp Ser Ser Thr Asp Val Asn tat etc aat gaa caa gge atc acg tgt tge gtg aat cat aat gge ttt 816 Tyr Leu Asn Glu Gln Gly Ile Thr Cys Cys Val Asn His Asn Gly Phe cgt ttt tgg ggc tta cgc acg act gca gaa gat cca tta ttc aag ttt 864 Arg Phe Trp Gly Leu Arg Thr Thr Ala Glu Asp Pro Leu Phe Lys Phe gaa gtg tac acc cgc act gca caa atc tta aaa gat acg att gca ggg 912 Glu Val Tyr Thr Arg Thr Ala Gln Ile Leu Lys Asp Thr Ile Ala Gly 295 gcg ttt gat tgg gca gtg gat aaa gat att tct gtc acg cta gtg aaa 960

Ala Phe Asp Trp Ala Val Asp Lys Asp Ile Ser Val Thr Leu Val Lys 305 gat att att gaa gca atc aat gcg aag tgg cgt gat tac acc aca aaa Asp Ile Ile Glu Ala Ile Asn Ala Lys Trp Arg Asp Tyr Thr Thr Lys ggc tac tta att ggc ggt aaa gcg tgg ctt aat aaa gag ctt aac agt 1056 Gly Tyr Leu Ile Gly Gly Lys Ala Trp Leu Asn Lys Glu Leu Asn Ser 340 345 gca acg aat tta aaa gat gcg aag ttg ttg atc tct tat gat tat cac 1104 Ala Thr Asn Leu Lys Asp Ala Lys Leu Leu Ile Ser Tyr Asp Tyr His 360 cca gta cca ccg ctc gaa cag cta ggc ttt aat cag tac att tct gat 1152 Pro Val Pro Pro Leu Glu Gln Leu Gly Phe Asn Gln Tyr Ile Ser Asp 380 gaa tac ctt gtt gat ttt tca aat cgt tta gca tcg ta 1190 Glu Tyr Leu Val Asp Phe Ser Asn Arg Leu Ala Ser 390 <210> 115 <211> 396 <212> PRT <213> Pasteurella multocida <400> 115 Met Ser Glu Glu Tyr Leu His Gly Val Lys Val Thr Glu Ile Asn Gln Ala Ile Arg Thr Ile Gln Ser Leu Ser Thr Ala Val Ile Gly Ile Val Cys Thr Ala Asn Asp Ala Asp Asn Glu Thr Phe Pro Leu Asn Glu Pro 40 Val Leu Ile Thr Asn Val Ala Ala Ile Gly Lys Ala Gly Lys Gln Gly Thr Leu Ser Arg Ala Leu Asp Gly Ile Ser Asp Val Val Asn Cys Lys Val Ile Val Val Arg Val Gln Glu Ser Ala Gln Glu Asp Glu Glu Thr Lys Ala Ser Glu Met Asn Thr Ala Ile Ile Gly Thr Ile Thr Glu Glu Gly Gln Tyr Thr Gly Leu Lys Ala Leu Leu Ile Ala Lys Asn Lys Phe Gly Ile Lys Pro Arg Ile Leu Cys Val Pro Lys Phe Asp Thr Lys Glu Val Ala Thr Glu Leu Ala Ser Ile Ala Ala Lys Leu Asn Ala Phe 145 Ala Tyr Ile Ser Cys Gln Gly Cys Lys Thr Lys Glu Gln Ala Val Gln

170

Tyr Lys Arg Asn Phe Ser Gln Arg Glu Val Met Leu Ile Met Gly Asp 185 Phe Leu Ser Phe Asn Val Asn Thr Ser Lys Val Glu Ile Asp Tyr Ala 200 Val Thr Arg Ala Ala Ala Met Arg Ala Tyr Leu Asp Lys Glu Gln Gly 215 Trp His Thr Ser Ile Ser Asn Lys Gly Ile Asn Gly Val Ser Gly Val Thr Gln Pro Leu Tyr Phe Asp Ile Asn Asp Ser Ser Thr Asp Val Asn 250 Tyr Leu Asn Glu Gln Gly Ile Thr Cys Cys Val Asn His Asn Gly Phe Arg Phe Trp Gly Leu Arg Thr Thr Ala Glu Asp Pro Leu Phe Lys Phe Glu Val Tyr Thr Arg Thr Ala Gln Ile Leu Lys Asp Thr Ile Ala Gly Ala Phe Asp Trp Ala Val Asp Lys Asp Ile Ser Val Thr Leu Val Lys 305 310 Asp Ile Ile Glu Ala Ile Asn Ala Lys Trp Arg Asp Tyr Thr Thr Lys Gly Tyr Leu Ile Gly Gly Lys Ala Trp Leu Asn Lys Glu Leu Asn Ser 345 Ala Thr Asn Leu Lys Asp Ala Lys Leu Leu Ile Ser Tyr Asp Tyr His 360 Pro Val Pro Pro Leu Glu Gln Leu Gly Phe Asn Gln Tyr Ile Ser Asp Glu Tyr Leu Val Asp Phe Ser Asn Arg Leu Ala Ser 390 <210> 116 <211> 2204 <212> DNA <213> Pasteurella multocida <220> <223> unkK <220> <221> CDS <222> (1)..(2202) <400> 116 atg aat aaa aat cgc tat aaa ctc att ttt agt aaa act aaa ggc tgt 48 Met Asn Lys Asn Arg Tyr Lys Leu Ile Phe Ser Lys Thr Lys Gly Cys ctt gta cct gtt gct gaa acg att aat tct gca gta gga aat gcc tca 96 Leu Val Pro Val Ala Glu Thr Ile Asn Ser Ala Val Gly Asn Ala Ser 25

tca Ser	aaa Lys	gac Asp 35	Val	tct Ser	gac Asp	acc Thr	gag Glu 40	Ile	agt Ser	gct Ala	tct Ser	caa Gln 45	Pro	gcg Ala	ctc Leu	144
aac Asn	tcg Ser 50	Pro	ctt Leu	tcg Ser	acc Thr	ctt Leu 55	tct Ser	gta Val	tta Leu	gtc Val	aaa Lys 60	Thr	gca Ala	ttt Phe	aat Asn	192
ccg Pro 65	Val	tca Ser	aca Thr	ttg Leu	atg Met 70	tcg Ser	ttg Leu	act Thr	tgg Trp	aaa Lys 75	Glu	tac Tyr	gcc Ala	gtt Val	tta Leu 80	240
tta Leu	tta Leu	agt Ser	gtg Val	gtg Val 85	tct Ser	ttt Phe	cct Pro	ctt Leu	atg Met 90	gca Ala	caa Gln	gcc Ala	tct Ser	gat Asp 95	aca Thr	288
gat Asp	tca Ser	gtg Val	gta Val 100	caa Gln	aga Arg	aaa Lys	cct Pro	gaa Glu 105	tta Leu	act Thr	gat Asp	gtg Val	acg Thr 110	aat Asn	agc Ser	336
aac Asn	agc Ser	tat Tyr 115	cat His	gtg Val	gaa Glu	tta Leu	gat Asp 120	aga Arg	gag Glu	cat His	cat His	aaa Lys 125	ggg Gly	gag Glu	cat His	384
caa Gln	aca Thr 130	aaa Lys	atc Ile	aaa Lys	cat His	act Thr 135	gag Glu	aat Asn	aat Asn	gtc Val	atc Ile 140	att Ile	gtt Val	gat Asp	att Ile	432
gca Ala 145	Lys	cca Pro	aac Asn	caa Gln	aag Lys 150	ggc Gly	att Ile	tca Ser	gat Asp	aac Asn 155	cgt Arg	ttt Phe	aaa Lys	cac His	ttc Phe 160	480
aac Asn	atc Ile	cca Pro	aat Asn	ggg Gly 165	gcg Ala	gta Val	ttt Phe	aac Asn	aat Asn 170	agc Ser	gcc Ala	aag Lys	gaa Glu	aaa Lys 175	cgc Arg	528
tca Ser	cag Gln	tta Leu	gtg Val 180	ggg Gly	tat Tyr	ttg Leu	cca Pro	ggt Gly 185	aac Asn	cag Gln	aat Asn	tta Leu	acg Thr 190	gaa Glu	ggt Gly	576
agt Ser	gaa Glu	gca Ala 195	aaa Lys	gcg Ala	atc Ile	tta Leu	aat Asn 200	cag Gln	gtg Val	act Thr	gga Gly	ccg Pro 205	gat Asp	gcc Ala	agt Ser	624
aaa Lys	att Ile 210	gaa Glu	ggc Gly	gcc Ala	ctt Leu	gaa Glu 215	att Ile	tta Leu	gly ggg	caa Gln	aaa Lys 220	gcc Ala	gat Asp	ttg Leu	gtg Val	672
att Ile 225	gcg Ala	aac Asn	caa Gln	aat Asn	ggc Gly 230	att Ile	gtg Val	ctt Leu	aat Asn	999 Gly 235	gta Val	aaa Lys	acc Thr	att Ile	aat Asn 240	720
gcc Ala	aat Asn	cgt Arg	ttt Phe	gtg Val 245	gca Ala	aca Thr	acc Thr	agt Ser	agt Ser 250	acc Thr	att Ile	gat Asp	cct Pro	gag Glu 255	caa Gln	768
atg Met	cag Gln	tta Leu	aat Asn 260	gtc Val	acg Thr	caa Gln	ggt Gly	aca Thr 265	gtg Val	aca Thr	att Ile	Gly 999	gtg Val 270	gat Asp	gga Gly	816
ttt Phe	gcc Ala	aca Thr 275	gat Asp	ggc Gly	tta Leu	cct Pro	tat Tyr 280	ttg Leu	gat Asp	atc Ile	att Ile	gcc Ala 285	aaa Lys	aag Lys	att Ile	864

							aaa Lys									912
							aac Asn									960
caa Gln	gtg Val	aca Thr	gaa Glu	aag Lys 325	cat His	acc Thr	gct Ala	gag Glu	gca Ala 330	caa Gln	ggt Gly	gaa Glu	att Ile	gcg Ala 335	att Ile	1008
agc Ser	ggt Gly	gcg Ala	agt Ser 340	acc Thr	ggt Gly	gca Ala	atg Met	tac Tyr 345	ggt Gly	aaa Lys	aat Asn	atc Ile	aaa Lys 350	tta Leu	atc Ile	1056
							gta Val 360									1104
gag Glu	gcg Ala 370	gat Asp	att Ile	caa Gln	att Ile	gaa Glu 375	acc Thr	cat His	gag Glu	ggc Gly	gat Asp 380	gtt Val	gaa Glu	tta Leu	ggc Gly	1152
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gtt Val	aaa Lys	gcc Ala	aac Asn 420	aaa Lys	gcg Ala	gtc Val	gat Asp	att Ile 425	caa Gln	gca Ala	caa Gln	gaa Glu	aca Thr 430	aca Thr	gta Val	1296
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agt Ser	aag Lys 450	agt Ser	gtg Val	aat Asn	ctt Leu	gaa Glu 455	gat Asp	aac Asn	gcg Ala	aaa Lys	ctt Leu 460	att Ile	gct Ala	aat Asn	gag Glu	1392
							tta Leu									1440
aag Lys	aaa Lys	gtg Val	acg Thr	cta Leu 485	gat Asp	gct Ala	gat Asp	aat Asn	tta Leu 490	gtc Val	aat Asn	agt Ser	aaa Lys	gaa Glu 495	atc Ile	1488
tat Tyr	gcg Ala	tct Ser	agc Ser 500	gaa Glu	ctt Leu	gat Asp	att Ile	caa Gln 505	acc Thr	aaa Lys	ggt Gly	cgt Arg	gat Asp 510	ctt Leu	tta Leu	1536
ctt Leu	gag Glu	gat Asp 515	ggg Gly	gtt Val	aat Asn	caa Gln	cca Pro 520	ctg Leu	agt Ser	ttc Phe	tta Leu	aaa Lys 525	ggc Gly	gct Ala	tca Ser	1584
ttg Leu	tta Leu 530	gcg Ala	ccg Pro	gly ggg	ttt Phe	gtc Val 535	aac Asn	act Thr	9 9 9 Gly	cta Leu	att Ile 540	cac His	agt Ser	aac Asn	ggt Gly	1632

aat Asn 545	gcc Ala	aag Lys	ctc Leu	act Thr	ttt Phe 550	aaa Lys	gat Asp	gac Asp	acc Thr	agt Ser 555	ttt Phe	gtg Val	act Thr	gaa Glu	gga Gly 560	1680
aat Asn	aac Asn	ttt Phe	atc Ile	aca Thr 565	gca Ala	aaa Lys	gac Asp	aac Asn	tta Leu 570	gaa Glu	atc Ile	acg Thr	gca Ala	aaa Lys 575	aat Asn	1728
gtt Val	caa Gln	att Ile	gat Asp 580	caa Gln	gcg Ala	aaa Lys	aat Asn	att Ile 585	caa Gln	tta Leu	aac Asn	gcg Ala	aat Asn 590	atc Ile	acg Thr	1776
atc Ile	aat Asn	acc Thr 595	aag Lys	tct Ser	ggt Gly	ttt Phe	gtg Val 600	aat Asn	tac Tyr	ggt Gly	acc Thr	tta Leu 605	gca Ala	agt Ser	gct Ala	1824
caa Gln	aat Asn 610	tta Leu	acg Thr	att Ile	aat Asn	acc Thr 615	gaa Glu	caa Gln	ggc Gly	agc Ser	att Ile 620	tat Tyr	aac Asn	ata Ile	ggc Gly	1872
ggt Gly 625	atc Ile	ttg Leu	G1y 999	gcg Ala	ggt Gly 630	aaa Lys	agt Ser	ttg Leu	aat Asn	ctg Leu 635	agc Ser	gcg Ala	aaa Lys	aga Arg	gga Gly 640	1920
gaa Glu	aac Asn	caa Gln	gga Gly	gga Gly 645	tat Tyr	ctt Leu	att Ile	aat Asn	caa Gln 650	ggt Gly	aag Lys	agt Ser	cta Leu	ctc Leu 655	cat His	1968
tct Ser	gaa Glu	ggc	gcc Ala 660	atg Met	aac Asn	ctc Leu	aca Thr	gcg Ala 665	gat Asp	cgc Arg	acg Thr	gtg Val	tac Tyr 670	aat Asn	tta Leu	2016
ggg ggg	aat Asn	att Ile 675	ttt Phe	gct Ala	aaa Lys	ggt Gly	gac Asp 680	gcg Ala	acg Thr	atc Ile	aat Asn	gca Ala 685	aac Asn	gcg Ala	tta Leu	2064
att Ile	aat Asn 690	gat Asp	gtt Val	act Thr	ctc Leu	aca Thr 695	ggt Gly	cgt Arg	ctt Leu	gag Glu	tat Tyr 700	caa Gln	gat Asp	ctg Leu	aaa Lys	2112
aaa Lys 705	gat Asp	tat Tyr	acg Thr	cgt Arg	tat Tyr 710	tat Tyr	cgt Arg	atc Ile	aat Asn	gaa Glu 715	acg Thr	gca Ala	aaa Lys	cat His	ggt Gly 720	2160
tgg Trp	cat His	aat Asn	aac Asn	ttc Phe 725	tat Tyr	gaa Glu	tta Leu	aac Asn	gtc Val 730	gac Asp	aga Arg	gtt Val	tct Ser	tg		2204

<210> 117

<211> 734

<212> PRT

<213> Pasteurella multocida

<400> 117

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1 10 15

Leu Val Pro Val Ala Glu Thr Ile Asn Ser Ala Val Gly Asn Ala Ser 20 25 30

Ser Lys Asp Val Ser Asp Thr Glu Ile Ser Ala Ser Gln Pro Ala Leu 35 45

Asn Ser Pro Leu Ser Thr Leu Ser Val Leu Val Lys Thr Ala Phe Asn Pro Val Ser Thr Leu Met Ser Leu Thr Trp Lys Glu Tyr Ala Val Leu Leu Leu Ser Val Val Ser Phe Pro Leu Met Ala Gln Ala Ser Asp Thr Asp Ser Val Val Gln Arg Lys Pro Glu Leu Thr Asp Val Thr Asn Ser 105 Asn Ser Tyr His Val Glu Leu Asp Arg Glu His His Lys Gly Glu His Gln Thr Lys Ile Lys His Thr Glu Asn Asn Val Ile Ile Val Asp Ile 135 Ala Lys Pro Asn Gln Lys Gly Ile Ser Asp Asn Arg Phe Lys His Phe 150 Asn Ile Pro Asn Gly Ala Val Phe Asn Asn Ser Ala Lys Glu Lys Arg 170 Ser Gln Leu Val Gly Tyr Leu Pro Gly Asn Gln Asn Leu Thr Glu Gly Ser Glu Ala Lys Ala Ile Leu Asn Gln Val Thr Gly Pro Asp Ala Ser Lys Ile Glu Gly Ala Leu Glu Ile Leu Gly Gln Lys Ala Asp Leu Val Ile Ala Asn Gln Asn Gly Ile Val Leu Asn Gly Val Lys Thr Ile Asn Ala Asn Arg Phe Val Ala Thr Thr Ser Ser Thr Ile Asp Pro Glu Gln 245 250 Met Gln Leu Asn Val Thr Gln Gly Thr Val Thr Ile Gly Val Asp Gly Phe Ala Thr Asp Gly Leu Pro Tyr Leu Asp Ile Ile Ala Lys Lys Ile 280 Glu Gln Lys Gln Ala Ile Thr Lys Glu Arg Thr Gly Asn Ser Glu Thr 295 Asp Ile Thr Phe Val Ala Gly Asn Ser Lys Tyr Asp Leu Lys Thr His Gln Val Thr Glu Lys His Thr Ala Glu Ala Gln Gly Glu Ile Ala Ile Ser Gly Ala Ser Thr Gly Ala Met Tyr Gly Lys Asn Ile Lys Leu Ile Val Thr Asp Lys Gly Ala Gly Val Lys His Asp Gly Ile Ile Leu Ser Glu Ala Asp Ile Gln Ile Glu Thr His Glu Gly Asp Val Glu Leu Gly 375

.

Asn Thr Lys Asn Asn Gln Asn Glu Asn Tyr Ala Lys Ala His Ala Glu Gly Asn Phe Thr Val Lys Gly Gly Lys His Val Ile Ile Gly Lys Glu Val Lys Ala Asn Lys Ala Val Asp Ile Gln Ala Gln Glu Thr Thr Val 425 Arg Gln Asn Ala Lys Leu Thr Ala Lys Thr Ser Ala Lys Ile Thr Ala 440 Ser Lys Ser Val Asn Leu Glu Asp Asn Ala Lys Leu Ile Ala Asn Glu 455 Leu Ser Thr Thr Thr Asn Lys Leu Thr Asn Lys Gly Ser Ile Tyr Gly 470 Lys Lys Val Thr Leu Asp Ala Asp Asn Leu Val Asn Ser Lys Glu Ile Tyr Ala Ser Ser Glu Leu Asp Ile Gln Thr Lys Gly Arg Asp Leu Leu Leu Glu Asp Gly Val Asn Gln Pro Leu Ser Phe Leu Lys Gly Ala Ser Leu Leu Ala Pro Gly Phe Val Asn Thr Gly Leu Ile His Ser Asn Gly Asn Ala Lys Leu Thr Phe Lys Asp Asp Thr Ser Phe Val Thr Glu Gly 550 Asn Asn Phe Ile Thr Ala Lys Asp Asn Leu Glu Ile Thr Ala Lys Asn 570 Val Gln Ile Asp Gln Ala Lys Asn Ile Gln Leu Asn Ala Asn Ile Thr 585 Ile Asn Thr Lys Ser Gly Phe Val Asn Tyr Gly Thr Leu Ala Ser Ala Gln Asn Leu Thr Ile Asn Thr Glu Gln Gly Ser Ile Tyr Asn Ile Gly 615 Gly Ile Leu Gly Ala Gly Lys Ser Leu Asn Leu Ser Ala Lys Arg Gly Glu Asn Gln Gly Gly Tyr Leu Ile Asn Gln Gly Lys Ser Leu Leu His Ser Glu Gly Ala Met Asn Leu Thr Ala Asp Arg Thr Val Tyr Asn Leu 665 Gly Asn Ile Phe Ala Lys Gly Asp Ala Thr Ile Asn Ala Asn Ala Leu Ile Asn Asp Val Thr Leu Thr Gly Arg Leu Glu Tyr Gln Asp Leu Lys Lys Asp Tyr Thr Arg Tyr Tyr Arg Ile Asn Glu Thr Ala Lys His Gly 710

Trp His Asn Asn Phe Tyr Glu Leu Asn Val Asp Arg Val Ser
725 730

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Ser His Glu Val Ile Lys Ser Glu Val Asn Thr Asn Glu Lys Asn His

Cys Asn His

<210> 120 <211> 548 <212> DNA <213> Pasteurella multocida <220> <223> unkP <220> <221> CDS <222> (1) .. (546) <400> 120 atg cgt gca tat ctt gat aaa gaa cag ggc tgg cat acg tct att tca 48 Met Arg Ala Tyr Leu Asp Lys Glu Gln Gly Trp His Thr Ser Ile Ser 10 aat aaa ggc att aat ggc gtg agc ggt gtc aca caa cca ctc tat ttt 96 Asn Lys Gly Ile Asn Gly Val Ser Gly Val Thr Gln Pro Leu Tyr Phe gac att aac gac agc teg act gat gtg aac tat etc aat gaa caa gge 144 Asp Ile Asn Asp Ser Ser Thr Asp Val Asn Tyr Leu Asn Glu Gln Gly atc acg tgt tgc gtg aat cat aat ggc ttt cgt ttt tgg ggc tta cgc 192 Ile Thr Cys Cys Val Asn His Asn Gly Phe Arg Phe Trp Gly Leu Arg acg act gca gaa gat cca tta ttc aag ttt gaa gtg tac acc cgc act Thr Thr Ala Glu Asp Pro Leu Phe Lys Phe Glu Val Tyr Thr Arg Thr 65 gca caa atc tta aaa gat acg att gca ggg gcg ttt gat tgg gca gtg 288 Ala Gln Ile Leu Lys Asp Thr Ile Ala Gly Ala Phe Asp Trp Ala Val gat aaa gat att tot gto acg ota gtg aaa gat att att gaa gca atc 336 Asp Lys Asp Ile Ser Val Thr Leu Val Lys Asp Ile Ile Glu Ala Ile 105 aat gcg aag tgg cgt gat tac acc aca aaa ggc tac tta att ggc ggt 384 Asn Ala Lys Trp Arg Asp Tyr Thr Thr Lys Gly Tyr Leu Ile Gly Gly 120 aaa gcg tgg ctt aat aaa gag ctt aac agt gca acg aat tta aaa gat 432 Lys Ala Trp Leu Asn Lys Glu Leu Asn Ser Ala Thr Asn Leu Lys Asp 135 gcg aag ttg ttg atc tct tat gat tat cac cca gta cca ccg ctc gaa 480 Ala Lys Leu Leu Ile Ser Tyr Asp Tyr His Pro Val Pro Pro Leu Glu 150 cag cta ggc ttt aat cag tac att tct gat gaa tac ctt gtt gat ttt 528 Gln Leu Gly Phe Asn Gln Tyr Ile Ser Asp Glu Tyr Leu Val Asp Phe 170 tca aat cgt tta gca tcq ta 548

Ser Asn Arg Leu Ala Ser 180

<210> 121

<211> 182

<212> PRT

<213> Pasteurella multocida

<400> 121

Met Arg Ala Tyr Leu Asp Lys Glu Gln Gly Trp His Thr Ser Ile Ser 1 5 10 15

Asn Lys Gly Ile Asn Gly Val Ser Gly Val Thr Gln Pro Leu Tyr Phe
20 25 30

Asp Ile Asn Asp Ser Ser Thr Asp Val Asn Tyr Leu Asn Glu Gln Gly
35 40

Ile Thr Cys Cys Val Asn His Asn Gly Phe Arg Phe Trp Gly Leu Arg
50 55 60

Thr Thr Ala Glu Asp Pro Leu Phe Lys Phe Glu Val Tyr Thr Arg Thr 65 70 75 80

Ala Gln Ile Leu Lys Asp Thr Ile Ala Gly Ala Phe Asp Trp Ala Val 85 90 95

Asp Lys Asp Ile Ser Val Thr Leu Val Lys Asp Ile Ile Glu Ala Ile
100 105 110

Asn Ala Lys Trp Arg Asp Tyr Thr Thr Lys Gly Tyr Leu Ile Gly Gly
115 120 125

Lys Ala Trp Leu Asn Lys Glu Leu Asn Ser Ala Thr Asn Leu Lys Asp 130 135 140

Ala Lys Leu Leu Ile Ser Tyr Asp Tyr His Pro Val Pro Pro Leu Glu 145 150 155 160

Gln Leu Gly Phe Asn Gln Tyr Ile Ser Asp Glu Tyr Leu Val Asp Phe
165 170 175

Ser Asn Arg Leu Ala Ser 180

<210> 122

<211> 69

<212> DNA

<213> Actinobacillus pleuropneumoniae

<220>

<223> apvA-or1

<220>

<221> CDS

<222> (1)..(69)

<400> 122

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Met Phe Tyr Val Met Leu Ala Asn Arg Thr Ser Ile Ile Ser Ser Ile

1 5 10 15

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gat aag ttt aag ata ctt agc
                                                                    69
Asp Lys Phe Lys Ile Leu Ser
             20
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<213> Actinobacillus pleuropneumoniae
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Asp Lys Phe Lys Ile Leu Ser
             20
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                                                                   47
   Leu Ser Ile Leu Asn Leu Ser Ile Asp Glu Ile Ile Asp Val Leu
     1
                     5
ttg gca agc atg aca ta
                                                                   64
Leu Ala Ser Met Thr
                 20
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<211> 20
<212> PRT
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                                     10
Ala Ser Met Thr
             20
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<211> 653
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<220>
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<222> (1)..(651)

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<210> 127

<211> 217

<212> PRT

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<213> Actinobacillus pleuropneumoniae
<400> 127
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Leu Ile Ser Phe Pro Phe Ile Thr Phe Ala Ser Asn Val Asn Gly Ala 1 5 10 15

Glu Ile Gly Leu Gly Gly Ala Arg Glu Ser Ser Ile Tyr Tyr Ser Lys 20 25 30

His Lys Val Ala Thr Asn Pro Phe Leu Ala Leu Asp Leu Ser Leu Gly 35 40

Asn Phe Tyr Met Arg Gly Thr Ala Gly Ile Ser Glu Ile Gly Tyr Glu 50 60

Gln Ser Phe Thr Asp Asn Phe Ser Val Ser Leu Phe Val Asn Pro Phe 65 70 75 80

Asp Gly Phe Ser Ile Lys Gly Lys Asp Leu Leu Pro Gly Tyr Gln Ser

Ile Gln Thr Arg Lys Thr Gln Phe Ala Phe Gly Trp Gly Leu Asn Tyr 100 105 110

Asn Leu Gly Gly Leu Phe Gly Leu Asn Asp Thr Phe Ile Ser Leu Glu 115 120 125

Gly Lys Ser Gly Lys Arg Gly Ala Ser Ser Asn Val Ser Leu Leu Lys 130 135 140

Ser Phe Asn Met Thr Lys Asn Trp Lys Val Ser Pro Tyr Ile Gly Ser 145 150 155

Ser Tyr Tyr Ser Ser Lys Tyr Thr Asp Tyr Tyr Phe Gly Ile Lys Gln 165 170 175

Ser Glu Leu Gly Asn Lys Ile Thr Ser Val Tyr Lys Pro Lys Ala Ala 180 185 190

Tyr Ala Thr His Ile Gly Ile Asn Thr Asp Tyr Ala Phe Thr Asn Asn 195 200 205

Leu Gly Met Gly Leu Ser Val Gly Trp 210 215

<210> 128

<211> 242

<212> DNA

<213> Actinobacillus pleuropneumoniae

<220>

<223> apvC

<220>

<221> CDS

<222> (1)..(240)

<400> 128

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Met Ala Arg Gln Ile Leu Ser Ala Ala Glu Leu Leu Ile Ala Lys Glu
ggt ttg caa aat tta tcg atg agg aaa atc gca agt gaa gcc ggt atc
Gly Leu Gln Asn Leu Ser Met Arg Lys Ile Ala Ser Glu Ala Gly Ile
gca aca ggc acg ctt tat ctc tat ttc aaa acg aaa gac gag tta ctg
                                                                   192
Ala Thr Gly Thr Leu Tyr Leu Tyr Phe Lys Thr Lys Asp Glu Leu Leu
     50
gat tgt ttg gcg gaa caa tta cat gaa cga tat tat cgt tat ctg aat
                                                                   240
Asp Cys Leu Ala Glu Gln Leu His Glu Arg Tyr Tyr Arg Tyr Leu Asn
                                          75
at
                                                                   242
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Met Ala Arg Gln Ile Leu Ser Ala Ala Glu Leu Leu Ile Ala Lys Glu
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Asn Ile Gln Lys Thr Val Ile Ala Ser Gly Thr Leu Gln Ala Thr Glu
caa gta gat att ggt gca caa gta tot ggg cag att aag cat att tta
Gln Val Asp Ile Gly Ala Gln Val Ser Gly Gln Ile Lys His Ile Leu
gta caa gaa gga cag aag gtt aaa aaa ggt gag cta tta gct gta att
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Val	Gln	Glu 35	Gly	Gln	Lys	Val	Lys 40	Lys	Gly	Glu	Leu	Leu 45	Ala	Val	Ile	
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ctg Leu	caa Gln	tca Ser	gat Asp	tgg Trp 85	gaa Glu	cgt Arg	cat His	caa Gln	cgt Arg 90	ttg Leu	ata Ile	cga Arg	acc Thr	aat Asn 95	gcg Ala	288
aca Thr	agc Ser	caa Gln	aag Lys 100	gaa Glu	aca Thr	gaa Glu	gaa Glu	gca Ala 105	aaa Lys	agt Ser	aga Arg	tta Leu	aat Asn 110	acg Thr	gcc Ala	336
aaa Lys	gca Ala	gaa Glu 115	ctt Leu	caa Gln	att Ile	gcg Ala	caa Gln 120	aat Asn	aat Asn	cta Leu	gat Asp	atc Ile 125	gct Ala	aaa Lys	atc Ile	384
aga Arg	gtg Val 130	gaa Glu	aaa Lys	gct Ala	gaa Glu	acc Thr 135	gaa Glu	cta Leu	gga Gly	tat Tyr	aca Thr 140	gaa Glu	att Ile	cgt Arg	tct Ser	432
cca Pro 145	ctt Leu	gat Asp	gca Ala	aca Thr	gta Val 150	att Ile	tca Ser	gta Val	ttt Phe	gcg Ala 155	caa Gln	aat Asn	ggt Gly	caa Gln	act Thr 160	480
tta Leu	gtc Val	acc Thr	acc Thr	caa Gln 165	caa Gln	gta Val	cca Pro	gtg Val	ctg Leu 170	atg Met	aaa Lys	tta Leu	gct Ala	aat Asn 175	at	527
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Val	Gln	Glu 35	Gly	Gln	Lys	Val	Lys 40	Lys	Gly	Glu	Leu	Leu 45	Ala	Val	Ile	
Asp	Pro 50	Arg	Leu	Ala	Glu	Thr 55	Glu	Leu	Lys	Leu	Ala 60	Lys	Ala	Glu	Leu	
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Leu	Gln	Ser	Asp	Trp 85	Glu	Arg	His	Gln	Arg 90	Leu	Ile	Arg	Thr	Asn 95	Ala	
Thr	Ser	Gln	Lys 100	Glu	Thr	Glu	Glu	Ala 105	Lys	Ser	Arg	Leu	Asn 110	Thr	Ala	

Lys Ala Glu Leu Gln Ile Ala Gln Asn Asn Leu Asp Ile Ala Lys Ile Arg Val Glu Lys Ala Glu Thr Glu Leu Gly Tyr Thr Glu Ile Arg Ser 135 Pro Leu Asp Ala Thr Val Ile Ser Val Phe Ala Gln Asn Gly Gln Thr Leu Val Thr Thr Gln Gln Val Pro Val Leu Met Lys Leu Ala Asn 170 <210> 132 <211> 867 <212> DNA <213> Actinobacillus pleuropneumoniae <220> <223> atpG <220> <221> CDS <222> (1)..(864) <400> 132 atg gca ggt gcg aaa gag ata aga acc aaa att gca agt gtg aaa aat 48 Met Ala Gly Ala Lys Glu Ile Arg Thr Lys Ile Ala Ser Val Lys Asn act caa aaa atc acc aaa gca atg gaa atg gtt gct acc tct aaa atg 96 Thr Gln Lys Ile Thr Lys Ala Met Glu Met Val Ala Thr Ser Lys Met cgt aaa acg caa gag cgt atg gct gcc agt cgt cct tat tcg gaa aca Arg Lys Thr Gln Glu Arg Met Ala Ala Ser Arg Pro Tyr Ser Glu Thr atc cgt aag gtg att agc cat att gcg aaa gga agc att ggt tat aag 192 Ile Arg Lys Val Ile Ser His Ile Ala Lys Gly Ser Ile Gly Tyr Lys 50 cac ccg ttt tta act gaa cgt gat att aaa aaa gta ggc tat ctt gtc 240 His Pro Phe Leu Thr Glu Arg Asp Ile Lys Lys Val Gly Tyr Leu Val 65 70 gtt tcg acc gat cgc ggt tta tgc ggt ggc ctt aat atc aat tta ttc 288 Val Ser Thr Asp Arg Gly Leu Cys Gly Gly Leu Asn Ile Asn Leu Phe aaa gcg act ttg aat gaa ttt aaa acg tgg aaa gat aaa gac gtt agt 336 Lys Ala Thr Leu Asn Glu Phe Lys Thr Trp Lys Asp Lys Asp Val Ser 100 105 gtt gag ctt ggt tta gta ggg tcg aaa ggc gta agc ttt tac caa aat 384 Val Glu Leu Gly Leu Val Gly Ser Lys Gly Val Ser Phe Tyr Gln Asn cta ggc tta aac gtg aga tct caa gta acg gga tta ggc gat aat ccg 432 Leu Gly Leu Asn Val Arg Ser Gln Val Thr Gly Leu Gly Asp Asn Pro gaa atg gaa cgt atc gtg ggc gca gtt aat gaa atg att aat gcg ttc

Glu 145	Met	Glu	Arg	Ile	Val 150	Gly	Ala	Val	Asn	Glu 155	Met	Ile	Asn	Ala	Phe 160	
	aac Asn															528
aat Asn	acg Thr	atg Met	tca Ser 180	caa Gln	aaa Lys	cct Pro	gtt Val	atc Ile 185	gca Ala	cag Gln	tta Leu	ctt Leu	ccg Pro 190	tta Leu	cct Pro	576
aaa Lys	cta Leu	gat Asp 195	gac Asp	gat Asp	gaa Glu	tta Leu	gat Asp 200	acg Thr	aaa Lys	ggt Gly	tca Ser	tgg Trp 205	gat Asp	tat Tyr	att Ile	624
tat Tyr	gaa Glu 210	ccg Pro	aat Asn	cca Pro	caa Gln	gtt Val 215	tta Leu	ttg Leu	gat Asp	agt Ser	tta Leu 220	ctt Leu	gtt Val	cgt Arg	tat Tyr	672
tta Leu 225	gaa Glu	act Thr	cag Gln	gta Val	tac Tyr 230	caa Gln	gca Ala	gtt Val	gta Val	gat Asp 235	aac Asn	cta Leu	gct Ala	tct Ser	gaa Glu 240	720
caa Gln	gcc Ala	gct Ala	cga Arg	atg Met 245	gta Val	gcg Ala	atg Met	aaa Lys	gcc Ala 250	gca Ala	aca Thr	gat Asp	aat Asn	gcg Ala 255	ggt Gly	768
aca Thr	tta Leu	atc Ile	gat Asp 260	gaa Glu	tta Leu	caa Gln	tta Leu	gtg Val 265	tat Tyr	aac Asn	aaa Lys	gct Ala	cgc Arg 270	caa Gln	gca Ala	816
agc Ser	att Ile	aca Thr 275	aat Asn	gaa Glu	tta Leu	aac Asn	gaa Glu 280	att Ile	gtt Val	gcg Ala	ggt Gly	gcc Ala 285	gca Ala	gca Ala	att Ile	864
taa																867
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	0> 13 Ala		בות	Lvc	Clu	Tla	7 ~~	Th =	Tira	Tla	7.7 a	0	17a l	T	3	-
1	ALG	GIY	ALG	Бу 5	Giu	116	Arg	1111	10	iie.	АІа	ser	vai	15	Asn	
	Gln		20					25					30			
Arg	Lys	Thr 35	Gln	Glu	Arg	Met	Ala 40	Ala	Ser	Arg	Pro	Tyr 45	Ser	Glu	Thr	
	Arg 50					55					60			-	-	
65	Pro				70					75					80	
Val	Ser	Thr	Asp	Arg 85	Gly	Leu	Cys	Gly	Gly 90	Leu	Asn	Ile	Asn	Leu 95	Phe	

Lys Ala Thr Leu Asn Glu Phe Lys Thr Trp Lys Asp Lys Asp Val Ser 100 105 Val Glu Leu Gly Leu Val Gly Ser Lys Gly Val Ser Phe Tyr Gln Asn Leu Gly Leu Asn Val Arg Ser Gln Val Thr Gly Leu Gly Asp Asn Pro Glu Met Glu Arg Ile Val Gly Ala Val Asn Glu Met Ile Asn Ala Phe Arg Asn Gly Glu Val Asp Ala Val Tyr Val Ala Tyr Asn Arg Phe Glu Asn Thr Met Ser Gln Lys Pro Val Ile Ala Gln Leu Leu Pro Leu Pro 185 Lys Leu Asp Asp Asp Glu Leu Asp Thr Lys Gly Ser Trp Asp Tyr Ile 200 Tyr Glu Pro Asn Pro Gln Val Leu Leu Asp Ser Leu Leu Val Arg Tyr 215 Leu Glu Thr Gln Val Tyr Gln Ala Val Val Asp Asn Leu Ala Ser Glu 235 Gln Ala Ala Arg Met Val Ala Met Lys Ala Ala Thr Asp Asn Ala Gly Thr Leu Ile Asp Glu Leu Gln Leu Val Tyr Asn Lys Ala Arg Gln Ala 260 Ser Ile Thr Asn Glu Leu Asn Glu Ile Val Ala Gly Ala Ala Ala Ile <210> 134 <211> 534 <212> DNA <213> Actinobacillus pleuropneumoniae <220> <223> atpH <220> <221> CDS <222> (1)..(531) <400> 134 atg tca gaa tta agt aca gta gct cgc ccc tac gct aaa gca gct ttt Met Ser Glu Leu Ser Thr Val Ala Arg Pro Tyr Ala Lys Ala Ala Phe gat ttt gct tta gaa caa ggt cag ttg gac aaa tgg caa gaa atg tta Asp Phe Ala Leu Glu Gln Gly Gln Leu Asp Lys Trp Gln Glu Met Leu cag ttt tcg gca ttc gtt gct gaa aac gaa caa gtg gcg gaa tat att 144 Gln Phe Ser Ala Phe Val Ala Glu Asn Glu Gln Val Ala Glu Tyr Ile aat tot too ott goa ago ggt cag att tot gaa act tit ato aaa ato

Asn	Ser 50	Ser	Leu	Ala	Ser	Gly 55	Gln	Ile	Ser	Glu	Thr 60	Phe	Ile	Lys	Ile	
	ggc Gly															240
	gaa Glu															288
	tca Ser															336
	gca Ala															384
	gaa Glu 130															432
	agc Ser															480
	ggt Gly															528
ttg Leu	taa															534
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1				5					10					15		
Asp	Phe	Ala	Leu 20	Glu	Gln	Gly	Gln	2 =	Asp	_	_		Glu 30	Met	Leu	
Gln	Phe	Ser 35	Ala	Phe	Val	Ala	Glu 40	Asn	Glu	Gln	Val	Ala 45	Glu	Tyr	Ile	
Asn	Ser 50	Ser	Leu	Ala	Ser	Gly 55	Gln	Ile	Ser	Glu	Thr 60	Phe	Ile	Lys	Ile	
Cys 65	Gly	Asp	Gln	Leu	Asp 70	Gln	Tyr	Gly	Gln	Asn 75	Phe	Ile	Arg	Val	Met 80	
Ala	Glu	Asn	Lys	Arg 85	Leu	Ala	Val	Leu	Pro 90	Met	Val	Phe	Asp	Thr 95	Phe	
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Met Glu Lys Arg Leu Gly Gln Lys Val Arg Leu Thr Asn Gln Ile Asp
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Leu
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Met Gln Glu Glu Val Ala Asn Phe Ala Asp Pro Ala Asp Arg Ala Thr
             20
cag gaa gaa ttc agt ctt gaa tta aga aac cgt gac cgt gag cgt
                                                                   144
Gln Glu Glu Glu Phe Ser Leu Glu Leu Arg Asn Arg Asp Arg Glu Arg
aaa ttg ctt aag aag att gag caa acg tta aat agc att gcc gaa gac
                                                                   192
Lys Leu Leu Lys Lys Ile Glu Gln Thr Leu Asn Ser Ile Ala Glu Asp
                         55
gaa tac ggc tat tgc gaa act tgc ggt gtt gaa atc ggt tta cgt cgt
                                                                   240
Glu Tyr Gly Tyr Cys Glu Thr Cys Gly Val Glu Ile Gly Leu Arg Arg
                    70
                                         75
tta gaa gcg cgc ccg acc gcg gat atg tgt atc gat tgc aaa aca ctt
                                                                   288
Leu Glu Ala Arg Pro Thr Ala Asp Met Cys Ile Asp Cys Lys Thr Leu
gcg gaa atc cgt gaa aag caa atg ggc tta taa
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Ala Glu Ile Arg Glu Lys Gln Met Gly Leu
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Lys Leu Leu Lys Lys Ile Glu Gln Thr Leu Asn Ser Ile Ala Glu Asp
Glu Tyr Gly Tyr Cys Glu Thr Cys Gly Val Glu Ile Gly Leu Arg Arg
Leu Glu Ala Arg Pro Thr Ala Asp Met Cys Ile Asp Cys Lys Thr Leu
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					att Ile											192
act Thr 65	atc Ile	ggt Gly	gcc Ala	aac Asn	gcc Ala 70	cct Pro	tat Tyr	atc Ile	ggt Gly	tta Leu 75	tta Leu	gga Gly	acc Thr	gta Val	tta Leu 80	240
ggg Gly	atc Ile	tta Leu	ctt Leu	acc Thr 85	ttc Phe	tat Tyr	cat His	tta Leu	ggg Gly 90	cat His	tcc Ser	ggc Gly	ggt Gly	gat Asp 95	att Ile	288
gac Asp	gcc Ala	gca Ala	tcc Ser 100	att Ile	atg Met	gtt Val	cac His	ctt Leu 105	tcg Ser	ctt Leu	gca Ala	tta Leu	aaa Lys 110	gca Ala	acc Thr	336
gca Ala	gcc Ala	ggt Gly 115	atc Ile	tta Leu	gtc Val	gct Ala	att Ile 120	ccg Pro	gca Ala	atg Met	atg Met	ttc Phe 125	tac Tyr	agc Ser	ggt Gly	384
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		aaa Lys			caa Gln 150	taa										453
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Glu	Arg	Val 35	Leu	Phe	Tyr	Lys	Gln 40	Leu	Asp	Val	Thr	Lys 45	Tyr	Asp	Thr	
Leu	Gln 50	Asp	Leu	Glu	Ile	Asp 55	Thr	Thr	Arg	Asn	Leu 60	Thr	Thr	Ile	Ser	
Thr 65	Ile	Gly	Ala	Asn	Ala 70	Pro	Tyr	Ile	Gly	Leu 75	Leu	Gly	Thr	Val	Leu 80	

Gly Ile Leu Leu Thr Phe Tyr His Leu Gly His Ser Gly Gly Asp Ile Asp Ala Ala Ser Ile Met Val His Leu Ser Leu Ala Leu Lys Ala Thr 105 Ala Ala Gly Ile Leu Val Ala Ile Pro Ala Met Met Phe Tyr Ser Gly Phe Asn Arg Lys Val Asp Glu Ser Lys Leu Lys Trp Gln Ala Ile Gln Ala Arg Lys Ala Asn Gln <210> 142 <211> 720 <212> DNA <213> Actinobacillus pleuropneumoniae <220> <223> fkpA <220> <221> CDS <222> (1)..(717) <400> 142 atg tta aaa aat aaa ctt tct gtt ctt gca atc gta gcc ggt acg ttc Met Leu Lys Asn Lys Leu Ser Val Leu Ala Ile Val Ala Gly Thr Phe 5 gtt tca gct caa act gca ttt gca gcg gat caa aaa ttc att gac gat Val Ser Ala Gln Thr Ala Phe Ala Ala Asp Gln Lys Phe Ile Asp Asp tca tca tat gca gtc ggc gta ttg atg ggt aaa aat atc gaa ggc gtc 144 Ser Ser Tyr Ala Val Gly Val Leu Met Gly Lys Asn Ile Glu Gly Val 40 gtt gaa toa caa aaa gaa att ttt tot tat aac caa gat aaa atc ttg 192 Val Glu Ser Gln Lys Glu Ile Phe Ser Tyr Asn Gln Asp Lys Ile Leu gcg ggt gtc caa gat acc atc aaa aaa acc ggt aaa tta acc gat gaa 240 Ala Gly Val Gln Asp Thr Ile Lys Lys Thr Gly Lys Leu Thr Asp Glu gat cta caa aaa caa tta aaa tcg ctt gat act tat ctt gca agt caa 288 Asp Leu Gln Lys Gln Leu Lys Ser Leu Asp Thr Tyr Leu Ala Ser Gln 85 gaa agc aaa att gcg gcg gag aaa agc aaa gca acc gta gaa gcc ggt 336 Glu Ser Lys Ile Ala Ala Glu Lys Ser Lys Ala Thr Val Glu Ala Gly 100 aat aaa ttt cgt acc gac tac gaa aaa caa agc ggc gtg aaa aaa acc 384 Asn Lys Phe Arg Thr Asp Tyr Glu Lys Gln Ser Gly Val Lys Lys Thr 120 gct tcc ggt tta ctt tat aaa att gaa aaa gcc ggc acg ggc gaa tcg

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	Lys									tat Tyr 155						480
										cgc Arg						528
										att Ile						576
ttg Leu	aaa Lys	aaa Lys 195	ggc Gly	gga Gly	aaa Lys	atg Met	gaa Glu 200	atc Ile	gtc Val	gtt Val	ccg Pro	cct Pro 205	gaa Glu	ctt Leu	ggt Gly	624
tac Tyr	ggc Gly 210	gaa Glu	cgc Arg	caa Gln	gca Ala	ggt Gly 215	aag Lys	att Ile	ccg Pro	gca Ala	agt Ser 220	tca Ser	acc Thr	tta Leu	aaa Lys	672
ttc Phe 225	gag Glu	att Ile	gaa Glu	ttg Leu	tta Leu 230	gat Asp	ttc Phe	aaa Lys	gcg Ala	gcc Ala 235	gaa Glu	gcg Ala	aaa Lys	aaa Lys	taa	720
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Asp Gly Thr Val Phe Asp Ser Ser Tyr Asp Arg Gly Glu Pro Ile Glu 165 170 Phe Gln Leu Asn Gln Leu Ile Pro Gly Trp Ile Glu Ala Ile Pro Met Leu Lys Lys Gly Gly Lys Met Glu Ile Val Val Pro Pro Glu Leu Gly Tyr Gly Glu Arg Gln Ala Gly Lys Ile Pro Ala Ser Ser Thr Leu Lys Phe Glu Ile Glu Leu Leu Asp Phe Lys Ala Ala Glu Ala Lys Lys 230 <210> 144 <211> 290 <212> DNA <213> Actinobacillus pleuropneumoniae <220> <223> HI0379 <220> <221> CDS <222> (3)..(287) <400> 144 tg cat agc gtg aga ggt ccg ggc ggt tat caa ctc ggt aag caa 47 His Ser Val Arg Gly Pro Gly Gly Gly Tyr Gln Leu Gly Lys Gln cct gaa gag att agt gtg ggg atg att att gcg gcg gtg aat gaa aat 95 Pro Glu Glu Ile Ser Val Gly Met Ile Ile Ala Ala Val Asn Glu Asn ete gae gta ace aaa tgt aaa ggt age gge aae tgt age aaa aae tet 143 Leu Asp Val Thr Lys Cys Lys Gly Ser Gly Asn Cys Ser Lys Asn Ser cag tgc tta acc cat cat tta tgg gaa cgt tta gaa gaa caa atc ggt 191 Gln Cys Leu Thr His His Leu Trp Glu Arg Leu Glu Glu Gln Ile Gly gtg ttt tta aat acg att act tta gcg gaa ctt gtt gaa gaa cat tcg 239 Val Phe Leu Asn Thr Ile Thr Leu Ala Glu Leu Val Glu Glu His Ser 70 gat cac gat tgt gaa aaa gaa cat tgc cac gat cat tca cac aaa cat 287 Asp His Asp Cys Glu Lys Glu His Cys His Asp His Ser His Lys His 85 taa 290 <210> 145 <211> 95 <212> PRT <213> Actinobacillus pleuropneumoniae

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Asp	Val	Thr 35	Lys	Cys	Lys	Gly	Ser 40		Asn	Cys	Ser	Lys 45		Ser	Gln	
Cys	Leu 50	Thr	His	His	Leu	Trp 55	Glu	Arg	Leu	Glu	Glu 60	Gln	Ile	Gly	Val	
Phe 65	Leu	Asn	Thr	Ile	Thr 70	Leu	Ala	Glu	Leu	Val 75		Glu	His	Ser	Asp 80	
His	Asp	Cys	Glu	Lys 85	Glu	His	Сув	His	Asp 90	His	Ser	His	Lys	His 95		
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tct Ser	gaa Glu	agc Ser 35	cta Leu	aaa Lys	aat Asn	ggc Gly	gac Asp 40	acc Thr	gtt Val	cag Gln	tta Leu	atc Ile 45	ggc Gly	ttc Phe	ggt Gly	144
act Thr	ttt Phe 50	aaa Lys	gta Val	aac Asn	gag Glu	cgt Arg 55	aat Asn	gca Ala	cgt Arg	acg Thr	ggt Gly 60	cgt Arg	aac Asn	ccg Pro	cgt Arg	192
acc Thr 65	ggc Gly	gaa Glu	gaa Glu	atc Ile	aaa Lys 70	atc Ile	gca Ala	gca Ala	tct Ser	aaa Lys 75	gtg Val	ccg Pro	gcg Ala	ttt Phe	gtt Val 80	240
gca Ala	ggt Gly	aaa Lys	gca Ala	tta Leu 85	aaa Lys	gat Asp	tta Leu	gta Val	aaa Lys 90	taa						273
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. 125

120

gac cgt gac ggt aat ccg aca acg att aaa ttt gat tat gca att att Asp Arg Asp Gly Asn Pro Thr Thr Ile Lys Phe Asp Tyr Ala Ile Ile 135 140 gca gcc ggt tct cgt ccg att cag ctt ccg ttc att cca cac gaa gat 480 Ala Ala Gly Ser Arg Pro Ile Gln Leu Pro Phe Ile Pro His Glu Asp 150 ccg cgt gtg tgg gat tct acg gat gca ctt aaa tta aaa gaa gta ccc 528 Pro Arg Val Trp Asp Ser Thr Asp Ala Leu Lys Leu Lys Glu Val Pro 165 gaa aaa att act cat tat ggg cc 551 Glu Lys Ile Thr His Tyr Gly 180 <210> 149 <211> 183 <212> PRT <213> Actinobacillus pleuropneumoniae <400> 149 Met Ser Lys Glu Ile Lys Thr Gln Val Val Val Leu Gly Ala Gly Pro Ala Gly Tyr Ser Ala Ala Phe Arg Cys Ala Asp Leu Gly Leu Glu Thr Val Ile Val Glu Arg Tyr Ser Thr Leu Gly Gly Val Cys Leu Asn Val

Gly Cys Ile Pro Ser Lys Ala Leu Leu His Val Ala Lys Val Ile Glu 50 55 60

Glu Ala Lys His Ala Glu Lys Asn Gly Ile Thr Phe Gly Glu Pro Asn 65 70 75 80

Ile Asp Leu Asp Lys Val Arg Ala Gly Lys Glu Ala Val Val Ser Lys
85 90 95

Leu Thr Gly Gly Leu Ala Gly Met Ala Lys Ala Arg Lys Val Thr Val

Val Glu Gly Leu Ala Ala Phe Thr Asp Pro Asn Thr Leu Val Ala Arg 115 120 125

Asp Arg Asp Gly Asn Pro Thr Thr Ile Lys Phe Asp Tyr Ala Ile Ile 130 135 140

Ala Ala Gly Ser Arg Pro Ile Gln Leu Pro Phe Ile Pro His Glu Asp 145 150 155 160

Pro Arg Val Trp Asp Ser Thr Asp Ala Leu Lys Leu Lys Glu Val Pro 165 170 175

Glu Lys Ile Thr His Tyr Gly 180

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											aac Asn					720
		_			_						tta Leu		_	_		768
											aat Asn					816
											gac Asp					864
											gcg Ala 300					912
											aac Asn					960
ggt Gly	tac Tyr	ggt Gly	gaa Glu	gca Ala 325	aac Asn	cct Pro	gta Val	acc Thr	ggc Gly 330	gca Ala	aca Thr	tgt Cys	gac Asp	aaa Lys 335	gtt Val	1008
aaa Lys	ggt Gly	cgt Arg	aaa Lys 340	gca Ala	tta Leu	atc Ile	gct Ala	tgc Cys 345	tta Leu	gca Ala	ccg Pro	gat Asp	cgt Arg 350	cgt Arg	gtt Val	1056
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Asn Thr Asp Arg Gly Thr Lys Tyr Gly Ile Asn Arg Asn Ser Val Thr 50 60

Tyr Gly Val Phe Gly Gly Tyr Gln Ile Leu Asn Gln Asp Lys Leu Gly 65 70 75

Leu Ala Ala Glu Leu Gly Tyr Asp Tyr Phe Gly Arg Val Arg Gly Ser

85 90 95

Glu Lys Pro Asn Gly Lys Ala Asp Lys Lys Thr Phe Arg His Ala Ala 100 105 110

His Gly Ala Thr Ile Ala Leu Lys Pro Ser Tyr Glu Val Leu Pro Asp 115 120 125

Leu Asp Val Tyr Gly Lys Val Gly Ile Ala Leu Val Asn Asn Thr Tyr 130 140

Lys Thr Phe Asn Ala Ala Gln Glu Lys Val Lys Thr Arg Arg Phe Gln 145 150 155 160

Ser Ser Leu Ile Leu Gly Ala Gly Val Glu Tyr Ala Ile Leu Pro Glu 165 170 175

Leu Ala Ala Arg Val Glu Tyr Gln Trp Leu Asn Asn Ala Gly Lys Ala 180 185 190

Ser Tyr Ser Thr Leu Asn Arg Met Gly Ala Thr Asp Tyr Arg Ser Asp 195 200 205

Ile Ser Ser Val Ser Ala Gly Leu Ser Tyr Arg Phe Gly Gln Gly Ala 210 215 220

Ala Pro Val Ala Ala Pro Ala Val Glu Thr Lys Asn Phe Ala Phe Ser 225 230 235 240

Ser Asp Val Leu Phe Ala Phe Gly Lys Ser Asn Leu Lys Pro Ala Ala 245 250 255

Ala Thr Ala Leu Asp Ala Met Gln Thr Glu Ile Asn Asn Ala Gly Leu 260 265 270

Ser Asn Ala Ala Ile Gln Val Asn Gly Tyr Thr Asp Arg Ile Gly Lys 275 280 285

Glu Ala Ser Asn Leu Lys Leu Ser Gln Arg Arg Ala Glu Thr Val Ala 290 295 300

Asn Tyr Ile Val Ser Lys Gly Ala Pro Ala Ala Asn Val Thr Ala Val 305 310 315 320

Gly Tyr Gly Glu Ala Asn Pro Val Thr Gly Ala Thr Cys Asp Lys Val 325 330 335

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235

Gly Gln Gly Ala Val Ala Pro Val Val Glu Pro Glu Val Val Thr Lys

230

	c gca ne Ala														768
	aa cca ys Pro														816
	ac tta sn Leu 275														864
	gt atc rg Ile 90			_	_								_	_	912
	aa act lu Thr														960
	a act														1008
_	gt gat ys Asp	_	_			-		_			_	_		-	1056
	at cgt sp Arg 355														1104
atg ta Met	aa					,									1110
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Leu Asn Leu Ala Leu Lys Pro Ser Tyr Glu Val Leu Pro Asp Leu Asp Val Tyr Gly Lys Val Gly Ile Ala Val Val Arg Asn Asp Tyr Lys Lys 135 Tyr Gly Ala Glu Asn Thr Asn Glu Ser Thr Thr Lys Phe His Lys Leu 150 Lys Ala Ser Thr Ile Leu Gly Ala Gly Val Glu Tyr Ala Ile Leu Pro 165 170 Glu Leu Ala Ala Arg Val Glu Tyr Gln Tyr Leu Asn Lys Ala Gly Asn Leu Asn Lys Ala Leu Val Arg Ser Gly Thr Gln Asp Val Asp Phe Gln 195 200 Tyr Ala Pro Asp Ile His Ser Val Thr Ala Gly Leu Ser Tyr Arg Phe Gly Gln Gly Ala Val Ala Pro Val Val Glu Pro Glu Val Val Thr Lys Asn Phe Ala Phe Ser Ser Asp Val Leu Phe Asp Phe Gly Lys Ser Ser 250 Leu Lys Pro Ala Ala Ala Thr Ala Leu Asp Ala Ala Asn Thr Glu Ile Ala Asn Leu Gly Leu Ala Thr Pro Ala Ile Gln Val Asn Gly Tyr Thr 280 Asp Arg Ile Gly Lys Glu Ala Ser Asn Leu Lys Leu Ser Gln Arg Arg 295 Ala Glu Thr Val Ala Asn Tyr Leu Val Ser Lys Gly Gln Asn Pro Ala 310 Asn Val Thr Ala Val Gly Tyr Gly Glu Ala Asn Pro Val Thr Gly Ala Thr Cys Asp Ala Val Lys Gly Arg Lys Ala Leu Ile Ala Cys Leu Ala Pro Asp Arg Arg Val Glu Val Gln Val Gln Gly Ala Lys Asn Val Ala Met <210> 154 <211> 1076 <212> DNA

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gcg Ala	gaa Glu	gcg Ala 35	cgt Arg	atc Ile	ggc Gly	gat Asp	gcg Ala 40	tat Tyr	cgt Arg	att Ile	aca Thr	gaa Glu 45	aaa Lys	caa Gln	gcg Ala	144
cgt Arg	tac Tyr 50	gaa Glu	caa Gln	atc Ile	gat Asp	gca Ala 55	att Ile	aaa Lys	gcg Ala	gat Asp	gtt Val 60	atc Ile	gca Ala	caa Gln	tta Leu	192
acc Thr 65	gca Ala	caa Gln	gac Asp	gaa Glu	acc Thr 70	gtt Val	tct Ser	gaa Glu	ggt Gly	gcg Ala 75	att Ile	att Ile	gat Asp	att Ile	att Ile 80	240
acc Thr	gca Ala	tta Leu	gaa Glu	agt Ser 85	tct Ser	att Ile	gtt Val	cgc Arg	ggt Gly 90	cgt Arg	att Ile	att Ile	gcc Ala	ggc Gly 95	gaa Glu	288
ccg Pro	cgt Arg	att Ile	gac Asp 100	ggt Gly	cgt Arg	acg Thr	gta Val	gat Asp 105	acg Thr	gtt Val	cgt Arg	gca Ala	tta Leu 110	gac Asp	att Ile	336
tgc Cys	acc Thr	ggc Gly 115	gta Val	tta Leu	cct Pro	cgt Arg	acg Thr 120	cac His	ggt Gly	tct Ser	gca Ala	atc Ile 125	ttt Phe	act Thr	cgc Arg	384
ggt Gly	gaa Glu 130	aca Thr	caa Gln	gca Ala	tta Leu	gcg Ala 135	gtt Val	gca Ala	acc Thr	tta Leu	ggt Gly 140	act Thr	gag Glu	cgc Arg	gat Asp	432
gca Ala 145	caa Gln	att Ile	gtt Val	gac Asp	gaa Glu 150	tta Leu	acc Thr	ggc Gly	gag Glu	aaa Lys 155	tca Ser	gac Asp	cgt Arg	ttc Phe	tta Leu 160	480
ttc Phe	cac His	tat Tyr	aac Asn	ttc Phe 165	cct Pro	ccg Pro	tac Tyr	tct Ser	gtc Val 170	ggt Gly	gaa Glu	acc Thr	ggt Gly	cgt Arg 175	atc Ile	528
ggt Gly	tcg Ser	ccg Pro	aaa Lys 180	cgt Arg	cgt Arg	gaa Glu	atc Ile	ggc Gly 185	cac His	ggt Gly	cgt Arg	tta Leu	gcg Ala 190	aaa Lys	cgc Arg	576
ggt Gly	gta Val	tta Leu 195	gcg Ala	gta Val	atg Met	ccg Pro	act Thr 200	gct Ala	gaa Glu	gaa Glu	ttc Phe	ccg Pro 205	tat Tyr	gta Val	gtg Val	624
cgc Arg	gta Val 210	gta Val	tct Ser	gaa Glu	att Ile	acc Thr 215	gaa Glu	tca Ser	aac Asn	ggt Gly	tct Ser 220	tct Ser	tca Ser	atg Met	gct Ala	672
tcc Ser 225	gta Val	tgc Cys	ggc Gly	gca Ala	tct Ser 230	tta Leu	gcg Ala	tta Leu	atg Met	gac Asp 235	gca Ala	ggc Gly	gta Val	ccg Pro	att Ile 240	720
aaa Lys	gcg Ala	gcg Ala	gtt Val	gcg Ala	ggt Gly	atc Ile	gca Ala	atg Met	ggc Gly	tta Leu	gtg Val	aaa Lys	gaa Glu	gaa Glu	gaa Glu	768

245 250 255 aaa ttt gtg gtg ctt tca gac atc tta ggt gac gaa gac cat tta ggc 816 Lys Phe Val Val Leu Ser Asp Ile Leu Gly Asp Glu Asp His Leu Gly 265 gat atg gac ttc aaa gta gcc ggt acg cgt gaa ggt gta acc gca ctt 864 Asp Met Asp Phe Lys Val Ala Gly Thr Arg Glu Gly Val Thr Ala Leu 280 caa atg gat att aaa atc gaa ggt atc acg cct gaa att atg caa atc 912 Gln Met Asp Ile Lys Ile Glu Gly Ile Thr Pro Glu Ile Met Gln Ile gca tta aat caa gcg aaa ggt gcg cgt atg cac atc tta agc gtg atg 960 Ala Leu Asn Gln Ala Lys Gly Ala Arg Met His Ile Leu Ser Val Met 315 gaa caa gcg att cct gca cct cgt gcc gat att tcc gat ttt gcg cct 1008 Glu Gln Ala Ile Pro Ala Pro Arg Ala Asp Ile Ser Asp Phe Ala Pro 325 cgt att cat acg atg aag atc gat ccg aag aaa atc aaa gac gtg atc 1056 Arg Ile His Thr Met Lys Ile Asp Pro Lys Lys Ile Lys Asp Val Ile 345 ggt aaa ggc ggt gcg gtt at 1076 Gly Lys Gly Gly Ala Val 355 <210> 155 <211> 358 <212> PRT <213> Actinobacillus pleuropneumoniae <400> 155 Asn Ile Lys Glu Phe Val Lys Glu Ala Gly Lys Pro Arg Trp Asp Trp Val Ala Pro Glu Pro Asn Thr Ala Leu Ile Asn Gln Val Lys Ala Leu Ala Glu Ala Arg Ile Gly Asp Ala Tyr Arg Ile Thr Glu Lys Gln Ala Arg Tyr Glu Gln Ile Asp Ala Ile Lys Ala Asp Val Ile Ala Gln Leu Thr Ala Gln Asp Glu Thr Val Ser Glu Gly Ala Ile Ile Asp Ile Ile Thr Ala Leu Glu Ser Ser Ile Val Arg Gly Arg Ile Ile Ala Gly Glu Pro Arg Ile Asp Gly Arg Thr Val Asp Thr Val Arg Ala Leu Asp Ile

Cys Thr Gly Val Leu Pro Arg Thr His Gly Ser Ala Ile Phe Thr Arg

Gly Glu Thr Gln Ala Leu Ala Val Ala Thr Leu Gly Thr Glu Arg Asp

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Leu	Thr	Ala	Cys 20	Asn	Glu	Glu	Lys	Pro 25	Lys	Ala	Ala	Glu	Ala 30	Ala	Ala	
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aaa Lys	tta Leu	caa Gln	ggt Gly	aaa Lys 85	gac Asp	ggc Gly	ggt Gly	tac Tyr	gat Asp 90	gtt Val	atc Ile	gca Ala	cct Pro	tct Ser 95	aac Asn	288
tac Tyr	ttc Phe	gtt Val	tca Ser 100	aaa Lys	atg Met	gcg Ala	aaa Lys	gaa Glu 105	ggt Gly	atg Met	tta Leu	gcg Ala	gaa Glu 110	tta Leu	gat Asp	336
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aaa Lys	cct Pro 130	Tyr	gac Asp	caa Gln	ggt Gly	aac Asn 135	aaa Lys	tac Tyr	tct Ser	tta Leu	ccg Pro 140	caa Gln	tta Leu	tta Leu	ggt Gly	432
gca Ala 145	ccg Pro	ggt Gly	atc Ile	gca Ala	ttt Phe 150	aac Asn	tca Ser	aat Asn	gac Asp	tat Tyr 155	aag Lys	ggc Gly	gat Asp	gcg Ala	ttc Phe 160	480
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										att Ile						576
ggt Gly	aaa Lys	aac Asn 195	cct Pro	aat Asn	aca Thr	acc Thr	aat Asn 200	ccg Pro	gaa Glu	gag Glu	att Ile	aaa Lys 205	gcg Ala	gct Ala	tac Tyr	624
gaa Glu	gag Glu 210	tta Leu	aga Arg	aaa Lys	tta Leu	cgt Arg 215	cca Pro	aac Asn	gta Val	ctt Leu	tct Ser 220	ttc Phe	act Thr	tca Ser	gac Asp	672
aac Asn 225	cca Pro	gcg Ala	aac Asn	tca Ser	ttt Phe 230	atc Ile	gca Ala	ggt Gly	gaa Glu	gta Val 235	tct Ser	gta Val	ggt Gly	caa Gln	tta Leu 240	720
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atg Met	gtg Val	ttc Phe	cca Pro	aaa Lys	gaa Glu	ggt Gly	cct Pro	gta Val	ctt Leu	tgg Trp	gtt Val	gat Asp	acg Thr	tta Leu	gcc Ala	816

260 270 265 att ccg gcg aat gcg aaa aac aaa gaa aat gcg cat aag tta atc aac 864 Ile Pro Ala Asn Ala Lys Asn Lys Glu Asn Ala His Lys Leu Ile Asn 280 tac tta tta agc gca ccg gtt gcg gaa aaa tta acg tta gaa atc ggt 912 Tyr Leu Leu Ser Ala Pro Val Ala Glu Lys Leu Thr Leu Glu Ile Gly 295 tat ccg act tca aac gta gaa gcg tta aaa aca tta cca aaa gag att 960 Tyr Pro Thr Ser Asn Val Glu Ala Leu Lys Thr Leu Pro Lys Glu Ile 305 acc gaa gat ccg gca atc tat ccg aca gct gat gtg tta aaa gcg gca 1008 Thr Glu Asp Pro Ala Ile Tyr Pro Thr Ala Asp Val Leu Lys Ala Ala 325 caa tgg caa gac gat gta ggt aat gca atc gaa ctt tac gaa aaa ta 1055 Gln Trp Gln Asp Asp Val Gly Asn Ala Ile Glu Leu Tyr Glu Lys <210> 157 <211> 351 <212> PRT <213> Actinobacillus pleuropneumoniae Met Lys Lys Leu Ala Gly Leu Phe Ala Ala Gly Leu Ala Thr Val Ala Leu Thr Ala Cys Asn Glu Glu Lys Pro Lys Ala Ala Glu Ala Ala Ala Gln Pro Ala Ala Gly Thr Val His Leu Tyr Thr Trp Thr Glu Tyr Val Pro Glu Gly Leu Leu Asp Glu Phe Thr Lys Gln Thr Gly Ile Lys Val Glu Val Ser Ser Leu Glu Ser Asn Glu Thr Met Tyr Ala Lys Leu 65 Lys Leu Gln Gly Lys Asp Gly Gly Tyr Asp Val Ile Ala Pro Ser Asn Tyr Phe Val Ser Lys Met Ala Lys Glu Gly Met Leu Ala Glu Leu Asp His Ala Lys Leu Pro Val Ile Lys Glu Leu Asn Gln Asp Trp Leu Asn Lys Pro Tyr Asp Gln Gly Asn Lys Tyr Ser Leu Pro Gln Leu Leu Gly Ala Pro Gly Ile Ala Phe Asn Ser Asn Asp Tyr Lys Gly Asp Ala Phe 150 155 Thr Ser Trp Gly Asp Leu Trp Lys Pro Glu Phe Ala Asn Lys Val Gln

Leu Leu Asp Asp Ala Arg Glu Val Phe Asn Ile Ala Leu Leu Lys Leu

180 185 190 Gly Lys Asn Pro Asn Thr Thr Asn Pro Glu Glu Ile Lys Ala Ala Tyr Glu Glu Leu Arg Lys Leu Arg Pro Asn Val Leu Ser Phe Thr Ser Asp Asn Pro Ala Asn Ser Phe Ile Ala Gly Glu Val Ser Val Gly Gln Leu 230 Trp Asn Gly Ser Val Arg Ile Ala Lys Lys Glu Gln Ala Pro Val Asn Met Val Phe Pro Lys Glu Gly Pro Val Leu Trp Val Asp Thr Leu Ala 265 Ile Pro Ala Asn Ala Lys Asn Lys Glu Asn Ala His Lys Leu Ile Asn Tyr Leu Leu Ser Ala Pro Val Ala Glu Lys Leu Thr Leu Glu Ile Gly 295 Tyr Pro Thr Ser Asn Val Glu Ala Leu Lys Thr Leu Pro Lys Glu Ile Thr Glu Asp Pro Ala Ile Tyr Pro Thr Ala Asp Val Leu Lys Ala Ala 325 Gln Trp Gln Asp Asp Val Gly Asn Ala Ile Glu Leu Tyr Glu Lys <210> 158 <211> 525 <212> DNA <213> Actinobacillus pleuropneumoniae <220> <223> rpmF <220> <221> CDS <222> (1)..(522) <400> 158 atg caa aag gta aaa cta ccc ctc acc att gac cca tat aaa gac gct Met Gln Lys Val Lys Leu Pro Leu Thr Ile Asp Pro Tyr Lys Asp Ala cag cgt cga atg gat tac gaa ggc tac atc tca cgt agt ctg ctt aat Gln Arg Arg Met Asp Tyr Glu Gly Tyr Ile Ser Arg Ser Leu Leu Asn cgt ttg ggt gaa tct gtg agc aat gtg cta agc gat gca caa gtt act 144 Arg Leu Gly Glu Ser Val Ser Asn Val Leu Ser Asp Ala Gln Val Thr ctc tcg tta tat atc gat ccg caa cgc tta acc gtt att aaa ggt acg 192 Leu Ser Leu Tyr Ile Asp Pro Gln Arg Leu Thr Val Ile Lys Gly Thr gcg aca gtg gaa gtg gaa ttc gat tgc caa cga tgc ggt aac ccg ttt

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	ttc Phe															384
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	atg Met															624
	caa Gln 210															672
	tta Leu								_				_		_	720
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	tca Ser															816
	caa Gln															864
	atc Ile 290															912
	gta Val															960
	ggt Gly															1008
	gcg Ala															1056
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35 40 45

Val Pro His Ala Ile Ile Glu Gln Arg Phe Gly Leu Ala Ala Arg Gln 50 55 60

Asp Val Leu Ser Asp Glu Met Gln Arg Ala Phe Phe Asp Ala Val Ile 65 70 75 80

Ala Glu Lys Ile Asn Leu Ala Gly Arg Pro Thr Phe Thr Pro Asn Asn 90 95

Tyr Gln Pro Ser Gln Glu Phe Ser Phe Thr Ala Thr Phe Glu Val Phe 100 105 110

Pro Glu Val Glu Leu Lys Gly Leu Glu Asn Ile Glu Val Glu Lys Pro 115 120 125

Val Val Glu Ile Thr Glu Ala Asp Leu Asp Lys Met Ile Asp Val Leu 130 135 140

Arg Lys Gln Gln Ala Thr Trp Ala Glu Ser Gln Ala Ala Gln Ala 145 150 155 160

Glu Asp Arg Val Val Ile Asp Phe Val Gly Ser Val Asp Gly Glu Glu 165 170 175

Phe Glu Gly Gly Lys Ala Thr Asp Phe Thr Leu Ala Met Gly Gln Ser 180 185 190

Arg Met Ile Pro Gly Phe Glu Glu Gly Ile Val Gly His Lys Ala Gly 195 200 205

Glu Gln Phe Asp Ile Asp Val Thr Phe Pro Glu Glu Tyr His Ala Glu 210 215 220

Asn Leu Lys Gly Lys Ala Ala Lys Phe Ala Ile Thr Leu Lys Lys Val 225 230 235 240

Glu Asn Ile Val Leu Pro Glu Leu Thr Glu Glu Phe Val Lys Lys Phe 245 250 255

Gly Ser Ala Lys Thr Val Glu Asp Leu Arg Ala Glu Ile Lys Lys Asn 260 265 270

Met Gln Arg Glu Leu Lys Asn Ala Val Thr Ala Arg Val Lys Asn Gln 275 280 285

Val Ile Asn Gly Leu Ile Ala Gln Asn Glu Ile Glu Val Pro Ala Ala 290 295 300 Ala Val Ala Glu Glu Val Asp Val Leu Arg Arg Gln Ala Val Gln Arg Phe Gly Gly Lys Pro Glu Met Ala Ala Gln Leu Pro Ala Glu Leu Phe Glu Ala Asp Ala Lys Arg Arg Val Gln Val Gly Leu Leu Leu Ser Thr 345 Val Ile Gly Thr Asn Glu Leu Lys Val Asp Glu Lys Arg Val Glu Glu Thr Ile Ala Glu Ile Ala Ser Ala Tyr Glu Gln Pro Ala Glu Val Val 375 Ala His Tyr Ala Lys Asn Arg Gln Leu Thr Glu Asn Ile Arg Asn Val 395 Val Leu Glu Glu Gln Ala Val Glu Val Val Leu Ala Lys Ala Lys Val 405 410 Thr Glu Lys Ala Thr Ser Phe Asp Glu Val Met Ala Gln Gln Ala Gln 425 Gly <210> 162 <211> 316 <212> DNA <213> Actinobacillus pleuropneumoniae <220> <223> tRNA-glu <400> 162 aatattgcgc tcaaatggca aagcggagag catctttaaa tgttgtcccc atcgtctaga 60 ggcctaggac atcgcccttt cacggcggta accggggttc gaatccccgt ggggacgcca 120 tttaaagatg acttttgttg tctgaattgt tctttaaaaa attggaaaca agctgaaaac 180 tgagagattt tcgaaagaaa gtctgagtag taaaagataa gtaattatct tgaaaatctt 240 agctgaacaa aagcagctaa gtgtttagtt gaataaagta tcgcgttgaa tgcgttcaaa 300 taaaatttga aaatat 316 <210> 163 <211> 85 <212> DNA <213> Actinobacillus pleuropneumoniae <220> <223> tRNA-leu <400> 163 getetggtgg tggaattggt agacacgeta tettgagggg gtagtgteca taggatgtgc 60

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gtc ggt ttg ccg atc ggt ttt ctg gca ttt tta acc ggt aaa gga gag Val Gly Leu Pro Ile Gly Phe Leu Ala Phe Leu Thr Gly Lys Gly Gly 35 40 45	g 144 u								
att tta gag aat ccg cgt tta cat caa gta tta gat gtg att att aat Ile Leu Glu Asn Pro Arg Leu His Gln Val Leu Asp Val Ile Ile Ass 50 55 60	192 1								
atc ggt cgt tcc gta ccg ttt att att ttg tta gtc gtg ttg tta cct Ile Gly Arg Ser Val Pro Phe Ile Ile Leu Leu Val Val Leu Leu Pro 65 70 75 80)								
Phe Thr Arg Leu Val Gly Thr Thr Leu Gly Thr Thr Ala Ala Ile 85 90 95	288								
gtg ccg tta agc gtt tcg gca att ccg ttt ttt gcg cgt tta act tca Val Pro Leu Ser Val Ser Ala Ile Pro Phe Phe Ala Arg Leu Thr Ser 100 105 110	a 336								
Asn Ala Leu Leu Glu Ile Pro Ala Gly Leu Thr Glu Ala Ala Lys Ser 115	384								
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tca ctg ccg att tta atc aat ggt atc aca tta act tta gtc gct tta Ser Leu Pro Ile Leu Ile Asn Gly Ile Thr Leu Thr Leu Val Ala Leu 145 150 155 160	ı								
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Asn Leu Ala Ile Ser Tyr Gly Glu His Arg Asn Met Val Tyr Val Lys	576 5								

623

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				cat His												336
				gtg Val												384
				att Ile												432
				tta Leu												480
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				atg Met												768

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Gln Ala Ala Arg Met Val Ala Met Lys Ala Ala Thr Asp Asn Ala Gly

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											aat Asn					576
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gga Gly 225	gcc Ala	ggt Gly	ccg Pro	ggc Gly	aat Asn 230	gaa Glu	gaa Glu	cga Arg	att Ile	gat Asp 235	gct Ala	tta Leu	gta Val	aaa Lys	gcg Ala 240	720
ggt Gly	gtc Val	gat Asp	gtg Val	cta Leu 245	tta Leu	atc Ile	gac Asp	tct Ser	tcg Ser 250	cac His	ggg ggg	cat His	tct Ser	gaa Glu 255	ggt Gly	768
gta Val	tta Leu	caa Gln	cgt Arg 260	gtg Val	cgt Arg	gaa Glu	acc Thr	cgt Arg 265	gca Ala	aaa Lys	tac Tyr	cct Pro	gat Asp 270	tta Leu	ccg Pro	816
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gat Asp	gca Ala 290	gga Gly	gcc Ala	agt Ser	gct Ala	gtg Val 295	aaa Lys	gta Val	gga Gly	atc Ile	ggc Gly 300	ccg Pro	ggt Gly	tca Ser	att Ile	912
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gct Ala	gat Asp	ggt Gly	gga Gly 340	att Ile	cgt Arg	tat Tyr	tca Ser	ggc Gly 345	gat Asp	att Ile	tca Ser	aaa Lys	gct Ala 350	att Ile	gcc Ala	1056
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gaa Glu	gcc Ala 370	ccg Pro	ggt Gly	gaa Glu	att Ile	gag Glu 375	ctt Leu	tat Tyr	caa Gln	ggc Gly	aga Arg 380	gca Ala	ttc Phe	aaa Lys	tcc Ser	1152
tac Tyr 385	cgt Arg	gga Gly	atg Met	gga Gly	tca Ser 390	tta Leu	ggt Gly	gca Ala	atg Met	agt Ser 395	aaa Lys	ggc Gly	tcg Ser	tca Ser	gat Asp 400	1200
cgc Arg	tat Tyr	ttc Phe	caa Gln	tct Ser 405	gat Asp	aat Asn	gcc Ala	gcc Ala	gac Asp 410	aag Lys	ctc Leu	gta Val	ccg Pro	gaa Glu 415	999 999	1248

att gaa ggg cgt atc gct tac aaa ggc tac ttg aaa gaa att atc cac 1296 Ile Glu Gly Arg Ile Ala Tyr Lys Gly Tyr Leu Lys Glu Ile Ile His 425 caa caa atg ggc ggc tta cgc tcc tgt atg gga tta acc ggc tgt gcc 1344 Gln Gln Met Gly Gly Leu Arg Ser Cys Met Gly Leu Thr Gly Cys Ala act att gaa gaa ctc cgc acc aaa gca gaa ttt gtc cgc att agt ggt 1392 Thr Ile Glu Glu Leu Arg Thr Lys A'a Glu Phe Val Arg Ile Ser Gly 455 gct ggt att aaa gaa agc cac gtc cac gat gtg aca att acc aaa gaa Ala Gly Ile Lys Glu Ser His Val His Asp Val Thr Ile Thr Lys Glu gca ccg aac tac cga atg ggt ta 1463 Ala Pro Asn Tyr Arg Met Gly 485

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<211> 487

<212> PRT

<213> Pasteurella (Mannheimia) haemolytica

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Val Pro Ala His Ser Thr Val Leu Pro Asn Thr Ala Asp Leu Ser Thr 20 25 30

Gln Leu Thr Lys Thr Ile Arg Leu Asn Ile Pro Met Leu Ser Ala Ala 35 40 45

Met Asp Thr Val Thr Glu Thr Lys Leu Ala Ile Ser Leu Ala Gln Glu 50 55

Gly Gly Ile Gly Phe Ile His Lys Asn Met Ser Ile Glu Arg Gln Ala 65 70 75 80

Asp Arg Val Arg Lys Val Lys Lys Phe Glu Ser Gly Ile Val Ser Glu 85 90 95

Pro Val Thr Ile Ser Pro Asp Met Thr Leu Ala Glu Leu Ala Glu Leu 100 105 110

Val Lys Lys Asn Gly Phe Ala Gly Tyr Pro Val Ile Asp Glu Asn Gln 115 120 125

Asn Leu Val Gly Ile Ile Thr Gly Arg Asp Thr Arg Phe Val Thr Asp 130 135 140

Leu Ser Lys Thr Val Arg Glu Phe Met Thr Pro Lys Asp Arg Leu Val 145 150 155 160

Thr Val Lys Glu Asn Ala Ser Arg Glu Glu Ile Phe His Leu Met His
165 170 175

Glu His Arg Val Glu Lys Val Leu Val Val Asn Asn Glu Phe Gln Leu 180 185 190

Lys Gly Met Ile Thr Leu Lys Asp Tyr Gln Lys Ala Glu Ser Lys Pro 195 200 205

Asn Ala Cys Lys Asp Glu Phe Gly Arg Leu Arg Val Gly Ala Ala Val 210 215 220

Gly Ala Gly Pro Gly Asn Glu Glu Arg Ile Asp Ala Leu Val Lys Ala 225 230 235 240

Gly Val Asp Val Leu Leu Ile Asp Ser Ser His Gly His Ser Glu Gly
245 250 255

Val Leu Gln Arg Val Arg Glu Thr Arg Ala Lys Tyr Pro Asp Leu Pro 260 265 270

Ile Val Ala Gly Asn Ile Ala Thr Ala Glu Gly Ala Ile Ala Leu Ala 275 280 285

Asp Ala Gly Ala Ser Ala Val Lys Val Gly Ile Gly Pro Gly Ser Ile 290 295 300

Cys Thr Thr Arg Ile Val Thr Gly Val Gly Val Pro Gln Ile Thr Ala 305 310 315 320

Ile Ala Glu Ala Ala Ala Leu Lys Glu Arg Gly Ile Pro Val Ile 325 330 335

Ala Asp Gly Gly Ile Arg Tyr Ser Gly Asp Ile Ser Lys Ala Ile Ala 340 345 350

Ala Gly Ala Ser Cys Val Met Val Gly Ser Met Phe Ala Gly Thr Glu 355 360 365

Glu Ala Pro Gly Glu Ile Glu Leu Tyr Gln Gly Arg Ala Phe Lys Ser 370 375 380

Tyr Arg Gly Met Gly Ser Leu Gly Ala Met Ser Lys Gly Ser Ser Asp 385 390 395

Arg Tyr Phe Gln Ser Asp Asn Ala Ala Asp Lys Leu Val Pro Glu Gly 405 410 415

Ile Glu Gly Arg Ile Ala Tyr Lys Gly Tyr Leu Lys Glu Ile Ile His
420 425 430

Gln Gln Met Gly Gly Leu Arg Ser Cys Met Gly Leu Thr Gly Cys Ala 435 440 445

Thr Ile Glu Glu Leu Arg Thr Lys Ala Glu Phe Val Arg Ile Ser Gly 450 455 460

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gcg Ala 225	ggc Gly	aaa Lys	cca Pro	cgt Arg	tgg Trp 230	gat Asp	tgg Trp	gtt Val	gca Ala	cca Pro 235	gag Glu	cca Pro	aat Asn	aca Thr	gat Asp 240	720
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tat Tyr	cgt Arg	atc Ile	gta Val 260	gaa Glu	aaa Lys	caa Gln	gtt Val	cgt Arg 265	tac Tyr	gag Glu	caa Gln	atc Ile	gat Asp 270	gcg Ala	att Ile	816
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gaa Glu	999 Gly 290	act Thr	atc Ile	atc Ile	gac Asp	atc Ile 295	atc Ile	acc Thr	gca Ala	tta Leu	gag Glu 300	agc Ser	caa Gln	atc Ile	gtg Val	912
cgt Arg 305	agc Ser	cgt Arg	att Ile	att Ile	gca Ala 310	Gly	gaa Glu	cca Pro	cgc Arg	att Ile 315	gac Asp	ggc Gly	cgt Arg	acg Thr	gtg Val 320	960
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cac His	ggt Gly	tct Ser	gct Ala 340	ctt Leu	ttc Phe	acc Thr	cgt Arg	ggc Gly 345	gaa Glu	acc Thr	caa Gln	gca Ala	tta Leu 350	gca Ala	gta Val	1056
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										tta Leu						1248
										gtg Val						1296
tct Ser	aac Asn	ggt Gly 435	tct Ser	tct Ser	tca Ser	atg Met	gca Ala 440	tct Ser	gtg Val	tgt Cys	ggt Gly	gcg Ala 445	tct Ser	ctt Leu	gcg Ala	1344
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atg Met 465	ggg Gly	ctc Leu	gtg Val	aaa Lys	gaa Glu 470	gac Asp	gag Glu	aaa Lys	ttc Phe	gtg Val 475	gta Val	ctt Leu	tct Ser	gac Asp	atc Ile 480	1440

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					atg Met											1584
aga Arg	atg Met 530	cac His	att Ile	tta Leu	ggt Gly	gta Val 535	atg Met	gaa Glu	caa Gln	gcc Ala	att Ile 540	ccg Pro	gca Ala	cct Pro	cgt Arg	1632
					tat Tyr 550											1680
					gat Asp											1728
gct Ala	tta Leu	acc Thr	gaa Glu 580	gag Glu	acc Thr	aat Asn	act Thr	tct Ser 585	atc Ile	gac Asp	att Ile	gat Asp	gat Asp 590	gac Asp	ggt Gly	1776
					gca Ala											1824
					atc Ile											1872
					cgt Arg 630											1920
					ggt Gly											1968
cgt Arg					gcg Ala											2016
gtg Val	aaa Lys	gtg Val 675	gta Val	gaa Glu	att Ile	gac Asp	cgt Arg 680	caa Gln	gga Gly	cgc Arg	att Ile	cgt Arg 685	ctg Leu	acg Thr	atg Met	2064
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<212> PRT

<213> Pasteurella (Mannheimia) haemolytica

<400> 171

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Val Lys Glu Gly Gln Asp Phe Phe Pro Leu Thr Val Asp Tyr Gln Glu
50 55 60

Arg Thr Tyr Ala Ala Gly Arg Ile Pro Gly Gly Phe Phe Lys Arg Glu 65 70 75 80

Gly Arg Pro Ser Glu Gly Glu Thr Leu Ile Ala Arg Leu Ile Asp Arg 85 90 95

Pro Val Arg Pro Leu Phe Pro Glu Gly Phe Phe Asn Glu Ile Gln Val

Ile Ala Thr Val Val Ser Val Asn Pro Gln Ile Ser Pro Asp Leu Val 115 120 125

Ala Met Ile Gly Ala Ser Ala Ala Leu Ser Leu Ser Gly Val Pro Phe 130 140

Asn Gly Pro Ile Gly Ala Ala Arg Val Gly Phe Ile Asn Asp Gln Phe 145 150 155 160

Val Leu Asn Pro Thr Thr Ser Glu Gln Lys Ile Ser Arg Leu Asp Leu 165 170 175

Val Val Ser Gly Thr Asp Lys Ala Val Leu Met Val Glu Ser Glu Ala 180 185 190

Asp Ile Leu Thr Glu Glu Gln Met Leu Ala Ala Val Val Phe Gly His 195 200 205

Glu Gln Gln Val Val Ile Glu Asn Ile Lys Glu Phe Val Lys Glu 210 215 220

Ala Gly Lys Pro Arg Trp Asp Trp Val Ala Pro Glu Pro Asn Thr Asp 225 230 235 240

Leu Ile Asn Lys Val Lys Ala Leu Ala Glu Thr Arg Leu Gly Asp Ala
245 250 255

Tyr Arg Ile Val Glu Lys Gln Val Arg Tyr Glu Gln Ile Asp Ala Ile 260 265 270

Lys Ala Glu Val Ile Ala Gln Leu Thr Ala Glu Asp Glu Thr Val Ser 275 280 285

Glu Gly Thr Ile Ile Asp Ile Ile Thr Ala Leu Glu Ser Gln Ile Val 290 295 300

Arg Ser Arg Ile Ile Ala Gly Glu Pro Arg Ile Asp Gly Arg Thr Val 305 310 315

Asp Thr Val Arg Ala Leu Asp Ile Cys Thr Ser Val Leu Pro Arg Thr 325 His Gly Ser Ala Leu Phe Thr Arg Gly Glu Thr Gln Ala Leu Ala Val Ala Thr Leu Gly Thr Glu Arg Asp Ala Gln Ile Ile Asp Glu Leu Thr Gly Glu Lys Ser Asp Arg Phe Leu Phe His Tyr Asn Phe Pro Pro Tyr 380 Ser Val Gly Glu Thr Gly Arg Ile Gly Ser Pro Lys Arg Arg Glu Ile Gly His Gly Arg Leu Ala Lys Arg Gly Val Leu Ala Val Met Pro Thr 410 Ala Glu Glu Phe Pro Tyr Val Val Arg Val Val Ser Glu Ile Thr Glu 425 Ser Asn Gly Ser Ser Ser Met Ala Ser Val Cys Gly Ala Ser Leu Ala 440 Leu Met Asp Ala Gly Val Pro Ile Lys Ala Ala Val Ala Gly Ile Ala Met Gly Leu Val Lys Glu Asp Glu Lys Phe Val Val Leu Ser Asp Ile Leu Gly Asp Glu Asp His Leu Gly Asp Met Asp Phe Lys Val Ala Gly Thr Arg Thr Gly Val Thr Ala Leu Gln Met Asp Ile Lys Ile Glu Gly 505 Ile Thr Pro Glu Ile Met Arg Ile Ala Leu Asn Gln Ala Lys Gly Ala Arg Met His Ile Leu Gly Val Met Glu Gln Ala Ile Pro Ala Pro Arg Ala Asp Ile Ser Asp Tyr Ala Pro Arg Ile His Thr Met Lys Ile Asp Pro Lys Lys Ile Lys Asp Val Ile Gly Lys Gly Gly Ala Thr Ile Arg Ala Leu Thr Glu Glu Thr Asn Thr Ser Ile Asp Ile Asp Asp Asp Gly 585 Thr Val Lys Ile Ala Ala Thr Asp Gly Asn Ala Ala Lys Ala Val Met Ala Arg Ile Glu Glu Ile Val Ala Glu Val Glu Val Asn Gln Ile Tyr Asn Gly Lys Val Thr Arg Val Val Asp Phe Gly Ala Phe Val Ser Ile 635 Leu Gly Gly Lys Glu Gly Leu Val His Ile Ser Gln Ile Thr Asn Glu

Arg Val Glu Arg Val Ala Asp Tyr Leu Thr Val Gly Gln Glu Val Gln 660 665 Val Lys Val Val Glu Ile Asp Arg Gln Gly Arg Ile Arg Leu Thr Met Lys Asp Ile Asn Asn Thr Asn Glu Ala Asn Ala Glu Glu Thr Val Ala Glu Asn Val Val Glu Thr Glu Gln Glu Asn Asn Phe <210> 172 <211> 1517 <212> DNA <213> Pasteurella (Mannheimia) haemolytica <220> <221> CDS <222> (1)..(1515) <220> <223> purF <400> 172 atg tgc ggc att gtc ggt att att ggg aat tcg ccg gtg aat cag gcg Met Cys Gly Ile Val Gly Ile Ile Gly Asn Ser Pro Val Asn Gln Ala 10 att tat gat ggt tta aca tta ctt caa cac cga gga caa gat gcc gca 96 Ile Tyr Asp Gly Leu Thr Leu Leu Gln His Arg Gly Gln Asp Ala Ala 25 ggt atc gtc acc ata gac gat gaa aat cgt ttc cgc tta cgc aaa gct 144 Gly Ile Val Thr Ile Asp Asp Glu Asn Arg Phe Arg Leu Arg Lys Ala 40 aac ggc tta gtc agc gat gtt ttc cag caa gag cat atg gtg aga tta 192 Asn Gly Leu Val Ser Asp Val Phe Gln Gln Glu His Met Val Arg Leu 50 caa ggc aat gtt gga att ggt cac gtt cgc tac cca aca gca ggt agc 240 Gln Gly Asn Val Gly Ile Gly His Val Arg Tyr Pro Thr Ala Gly Ser 65 tca agt gtg tct gaa gcc cag cca ttt tat gtc aat tca cct ttc ggt Ser Ser Val Ser Glu Ala Gln Pro Phe Tyr Val Asn Ser Pro Phe Gly 85 att acc tta gtt cac aac ggt aat tta act aat aat gcg gaa ctt aaa Ile Thr Leu Val His Asn Gly Asn Leu Thr Asn Asn Ala Glu Leu Lys 100 get ege tta tae aac gaa gee ege ege cat gtg aac aet aat tet gat Ala Arg Leu Tyr Asn Glu Ala Arg Arg His Val Asn Thr Asn Ser Asp 120 tot gaa too ott ott aat att ttt got tac ttt tta gat oto tat too 432 Ser Glu Ser Leu Leu Asn Ile Phe Ala Tyr Phe Leu Asp Leu Tyr Ser 135 act cag cat tta agc cca gac aat atc ttt gaa acg gtt cgt aaa acc 480

Thr 145	Gln	His	Leu	Ser	Pro 150	Asp	Asn	Ile	Phe	Glu 155	Thr	Val	Arg	Lys	Thr 160	
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ctg Leu	ggt Gly	aaa Lys 195	cgt Arg	gaa Glu	atc Ile	gag Glu	ggt Gly 200	aaa Lys	acc Thr	gaa Glu	tat Tyr	atg Met 205	ttt Phe	gct Ala	tcg Ser	624
gaa Glu	agt Ser 210	gtg Val	gct Ala	ctt Leu	gat Asp	gta Val 215	gtg Val	gly ggg	ttt Phe	gaa Glu	ttt Phe 220	gtg Val	cga Arg	gat Asp	gtg Val	672
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gtt Val	tat Tyr	ttt Phe	gcc Ala 260	cgt Arg	cct Pro	gat Asp	tcc Ser	gtc Val 265	att Ile	gat Asp	ggc Gly	gtt Val	tct Ser 270	Val	tat Tyr	816
tct Ser	gca Ala	cga Arg 275	gtg Val	cat His	atg Met	ggc Gly	gaa Glu 280	tta Leu	tta Leu	ggt Gly	gag Glu	aaa Lys 285	att Ile	aaa Lys	cgt Arg	864
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gaa Glu 305	acc Thr	tca Ser	aat Asn	gat Asp	att Ile 310	gcg Ala	gta Val	cgt Arg	att Ile	gct Ala 315	aat Asn	atg Met	ttg Leu	tat Tyr	aaa Lys 320	960
ccc Pro	tat Tyr	cgt Arg	Gln	999 Gly 325	Phe	gtt Val	aaa Lys	aac Asn	cgc Arg 330	tat Tyr	gta Val	gct Ala	cga Arg	act Thr 335	ttt Phe	1008
att Ile	atg Met	ccg Pro	999 Gly 340	caa Gln	gca Ala	cag Gln	cgt Arg	aaa Lys 345	agc Ser	tcg Ser	gtt Val	cgc Arg	cgt Arg 350	aaa Lys	tta Leu	1056
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gat Asp	tct Ser 370	att Ile	gta Val	cga Arg	ggt Gly	aca Thr 375	acg Thr	tct Ser	gaa Glu	caa Gln	atc Ile 380	gtg Val	gaa Glu	atg Met	gca Ala	1152
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gtg gat aaa ttg Val Asp Lys Leu 435	att ttc caa Ile Phe Glr	gac ctt gaa Asp Leu Glu 440	gca ctt tat aag Ala Leu Tyr Lys 445	tct att 1344 Ser Ile
caa ctg gaa aat Gln Leu Glu Asn 450	ccg act att Pro Thr Ile 455	His Arg Phe	gat gac tct gta Asp Asp Ser Val 460	ttt aca 1392 Phe Thr
gga gaa tat att Gly Glu Tyr Ile 465	aca ggt gat Thr Gly Asp 470	Val Asp Lys	tgc tat tta gac Cys Tyr Leu Asp 475 .	agt ata 1440 Ser Ile 480
gca aga tct cga Ala Arg Ser Arg	aac gat aaa Asn Asp Lys 485	gca aaa gca Ala Lys Ala 490	gag gcg gca aaa Glu Ala Ala Lys	caa gcc 1488 Gln Ala 495
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Asn Gly Leu Val Ser Asp Val Phe Gln Gln Glu His Met Val Arg Leu

Gln Gly Asn Val Gly Ile Gly His Val Arg Tyr Pro Thr Ala Gly Ser 65 70 75 80

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Ile Thr Leu Val His Asn Gly Asn Leu Thr Asn Asn Ala Glu Leu Lys 100 105 110

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PCT

(19) World Intellectual Property Organization

International Bureau



(43) International Publication Date 26 September 2002 (26.09.2002)

(10) International Publication Number WO 02/075507 A3

- C12N 1/20, (51) International Patent Classification7: A61K 39/102, 35/74, C12N 15/31, 15/63, C07K 14/285, 16/12, C12Q 1/18, G01N 33/68
- (21) International Application Number: PCT/US02/01971
- (22) International Filing Date: 17 January 2002 (17.01.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 09/809,665 15 March 2001 (15.03.2001) US
- (71) Applicant (for all designated States except US): PHAR-MACIA & UPJOHN COMPANY [US/US]; 301 Henrietta Street, Kalamazoo, MI 49007 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): LOWERY, David, E. [US/US]; 1207 Woodland Drive, Portage, MI 49024 (US). FULLER, Troy, E. [US/US]; 111 Dreamfield Drive, Battle Creek, MI 49014 (US). KENNEDY, Michael, J. [US/US]; 2364 Quincy Avenue, Portage, MI 49024 (US).
- (74) Agent: WILLIAMS, Joseph, A., Jr.; Marshall, Gerstein & Borun, 6300 Sears Tower, 233 South Wacker Drive, Chicago, IL 60606 (US).

- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



02/075507 A3

(54) Title: ANTI-BACTERIAL VACCINE COMPOSITIONS

(57) Abstract: Gram negative bacterial virulence genes are identified, thereby allowing the identification of anti-bacterial agents that target these virulence genes and their products, and the provision of gram negative bacterial mutants useful in vaccines.



International Application No
PCT/US 02/01971

A. CLASSI IPC 7	FICATION OF SUBJECT MATTER C12N1/20 A61K39/102 A61K35/ C07K14/285 C07K16/12 C12Q1/1	74 C12N15/31 8 G01N33/68	C12N15/63			
According to	o International Patent Classification (IPC) or to both national classific	ation and IPC				
B FIFLDS	SEARCHED					
	ocumentation searched (classification system followed by classification C12N A61K C07K C12Q G01N	on symbols)				
Documental	tion searched other than minimum documentation to the extent that s	such documents are included in the	fields searched			
Electronic da	ata base consulted during the international search (name of data ba	se and, where practical, search ter	ms used)			
EMBL,	EPO-Internal, WPI Data, BIOSIS, MED	LINE				
C. DOCUME	ENTS CONSIDERED TO BE RELEVANT					
Category °	Citation of document, with indication, where appropriate, of the rela	evant passages	Relevant to claim No.			
Χ	DATABASE EMBL [Online] 10 February 2001 (2001-02-10) MAY B.J. ET AL.: "Pasteurella mu PM70 section 152 of 204 of the co	ltocida omplete	1-41			
x	genome" Database accession no. AE006064 XP002224305 nucleotides 3352-4146 & DATABASE EMBL [Online] Entry AE006064, 10 February 2001 (2001-02-10) MAY B.J. ET AL.: "Pasteurella mu	Itocida	5-23,25, 28			
Α	PM70 section 31 of 204 of the congenome" the whole document & BARBARA J. MAY ET AL.: "Complegenomic sequence of Pasteurella Pm70" PROCEEDINGS OF THE NATIONAL ACAD	ete multocida, EMY OF	1-41			
		-/				
X Furth	ner documents are listed in the continuation of box C.	Patent family members a	re listed in annex.			
° Special cat	tegories of cited documents:	"T" later document nublished after	the international filing date			
conside	nt defining the general state of the art which is not ered to be of particular relevance	"T" later document published after or priority date and not in con cited to understand the princi invention	iffict with the application but ple or theory underlying the			
"E" earlier d	locument but published on or after the international ate	"X" document of particular relevan cannot be considered novel of	nce; the claimed invention			
"L" documer which i	nt which may throw doubts on priority claim(s) or is cited to establish the publication date of another n or other special reason (as specified)	involve an inventive step whe "Y" document of particular relevan cannot be considered to invol document is combined with o	en the document is taken alone			
other n		document is combined with o ments, such combination bein in the art.	ne or more other such docu- ng obvious to a person skilled			
"P" docume later th	nt published prior to the international filing date but an the priority date claimed	"&" document member of the same	e patent family			
Date of the a	actual completion of the international search	Date of mailing of the internati	ional search report			
12	2 May 2003	,	1 6. 05. 2003			
Name and m	nailing address of the ISA	Authorized officer				
	European Patent Office, P.B. 5818 Patentlaan 2					
	NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montero Lopez, B				



International Application No PCT/US 02/01971

Cantinu	AND DOCUMENTS CONSIDERED TO A TOTAL OF THE STATE OF THE S	PCT/US 02/01971
ategory °	ation) DOCUMENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages	
ulogory	onation of decament, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	SCIENCES OF USA, vol. 98, no. 6, 13 March 2001 (2001-03-13), pages 3460-3465, XP002202785 WASHINGTON US page 3463, right-hand column, paragraph 2 -page 3464, left-hand column, paragraph 1	
	COONEY ET AL: "Three contiguous lipoprotein genes in Pasteurella haemolytica A1 which are homologous to a lipoprotein gene in Haemophilus influenza Type b" INFECTION AND IMMUNITY, AMERICAN SOCIETY OF MICROBIOLOGY, WASHINGTON, DC, US, vol. 61, no. 11, November 1993 (1993-11), pages 4682-4688, XP002148894 ISSN: 0019-9567 abstract page 4683, left-hand column, last paragraph -page 4685, left-hand column, paragraph 1; figures 3,4 page 4686, right-hand column, paragraph 2	5-23,25, 28
	TROY E. FULLER ET AL.: "Identification of Pasteurella multocida virulence genes in a septicemic mouse model using signature-tagged mutagenesis" MICROBIAL PATHOGENESIS, vol. 29, 2000, pages 25-38, XP002224304 the whole document	1-41

INTERNATIONAL SEARCH REPORT

International application No. PCT/US 02/01971

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box If Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:
see additional sheet
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. X As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
1-41 partially
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark on Protest The additional search fees were accompanied by the applicant's protest. X No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-41 partially

Gram-negative bacteria comprising a mutation in a gene of sequence SEQ ID NO:1 resulting in decreased activity of the gene product; immunogenic composition comprising the bacteria; method of producing such mutant bacteria; nucleotide sequence comprising SEQ ID NO:1, vector and host cell comprising the same and use thereof to produce a polypeptide; encoded polypeptide of sequence SEQ ID NO:2; antibody against it; use of the polypeptide of sequence SEQ ID NO:2 for identifying antibacterial agents.

2. Claims: 1-41 partially

Idem as subject 1 for, respectively sequences SEQ ID NO:3 and 4; 7 and 8; 9 and 10; 21 and 22; 25 and 26.

3. Claims: 1-4, 21-23, 27, 28 partially

Gram-negative bacteria comprising a mutation in a gene of sequence SEQ ID NO:27 resulting in decreased activity of the gene product; immunogenic composition comprising the bacteria; nucleotide sequence comprising SEQ ID NO:27.

4. Claims: 1-41 partially

Idem as subject 1 for, respectively, sequences SEQ ID NOs:29 and 30; 39 and 40; 41 and 42; 51 and 52; 53 and 54; 55 and 56.

5. Claims: 1-28 partially

Gram-negative bacteria comprising a mutation in a gene of sequence SEQ ID NO:57 resulting in decreased activity of the gene product; immunogenic composition comprising the bacteria; method of producing such mutant bacteria; nucleotide sequence comprising SEQ ID NO:57.

6. Claims: 1-41 partially

Idem as subject 1 for, respectively sequences SEQ ID NOs:58 and 59; 60 and 61; 68 and 69; 72 and 73; 74 and 75; 76 and 77; 78 and 79; 80 and 81; 82 and 83; 84 and 85; 104 and 105; 108 and 109; 112 and 113; 116 and 117; 118 and 119; 120 and 121; 122 and 123; 124 and 125; 126 and 127; 128 and 129; 130 and 131

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

7. Claims: 5-26, 29-41 partially

Attenuated Pasteurellaceae bacteria comprising a mutation in a gene of sequence SEQ ID NO:11; immunogenic composition containing it; method of producing such mutant bacteria; nucleotide sequence comprising SEQ ID NO:11, vector and host cell comprising the same and use thereof to produce a polypeptide; encoded polypeptide of sequence SEQ ID NO:12; antibody against it; use of the polypeptide of sequence SEQ ID NO:12 for identifying antibacterial agents.

8. Claims: 5-26, 28-41 partially

Idem as subject 36 for, respectively, sequences SEQ ID NOs:13 and 14; 15 and 16; 17 and 18; 19 and 20; 23 and 24; 31 and 32; 33 and 34; 35 and 36; 37 and 38; 70 and 71; 100 and 101; 102 and 103; 106 and 107; 110 and 111; 114 and 115; 132 and 133; 134 and 135; 136 and 137; 138 and 139; 140 and 141; 142 and 143; 144 and 145; 146 and 147; 148 and 149; 150 and 151; 152 and 153; 154 and 155; 156 and 157; 158 and 159; 160 and 161

9. Claims: 5-26 partially

Attenuated Pasteurellaceae bacteria comprising a mutation in, respectively a gene of sequence SEQ ID NO:162 and 163; immunogenic composition containing it; method of producing such mutant bacteria; nucleotide sequence comprising SEQ ID NO:162 or 163.

10. Claims: 5-26, 28-41 partially

Idem as subject 36 for, respectively, sequences SEQ ID NOs:164 and 165; 166 and 167; 168 and 169; 170 and 171; 172 and 173; 174 and 175

(19) World Intellectual Property Organization International Bureau





(43) International Publication Date 26 September 2002 (26.09.2002)

PCT

(10) International Publication Number WO 02/075507 A3

- (51) International Patent Classification⁷: C12N 1/20, A61K 39/102, 35/74, C12N 15/31, 15/63, C07K 14/285, 16/12, C12Q 1/18, G01N 33/68
- (21) International Application Number: PCT/US02/01971
- **(22) International Filing Date:** 17 January 2002 (17.01.2002)
- (25) Filing Language:

English

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(30) Priority Data:

09/809,665

15 March 2001 (15.03.2001) US

- (71) Applicant (for all designated States except US): PHAR-MACIA & UPJOHN COMPANY [US/US]; 301 Henrictta Street, Kalamazoo, MI 49007 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): LOWERY, David, E. [US/US]; 1207 Woodland Drive, Portage, MI 49024 (US). FULLER, Troy, E. [US/US]; 111 Dreamfield Drive, Battle Creek, MI 49014 (US). KENNEDY, Michael, J. [US/US]; 2364 Quincy Avenue, Portage, MI 49024 (US).
- (74) Agent: WILLIAMS, Joseph, A., Jr.; Marshall, Gerstein & Borun, 6300 Sears Tower, 233 South Wacker Drive, Chicago, IL 60606 (US).

- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CII, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GII, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- with amended claims
- (88) Date of publication of the international search report:

12 September 2003

Date of publication of the amended claims: 11 December 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

02/075507 A3

(54) Title: ANTI-BACTERIAL VACCINE COMPOSITIONS

AMENDED CLAIMS

[received by the International Bureau on 11 July 2003 (11.07.03) original claims 1 to 41 have been amended by claims 1 to 29

WHAT IS CLAIMED IS:

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- 1. An attenuated *Mannheimia* bacteria comprising a mutation in a gene represented by a nucleotide sequence set forth in any one of SEQ ID NOS: 166, 168, 170, 172 and 174 or a species homolog thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.
- 2. The *Mannheimia* bacteria of claim 1 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.
- 10 3. The *Mannheimia* bacteria of claim 1 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.
 - 4. The *Mannheimia* bacteria of claim 1 wherein said mutation results in deletion of all or part of said gene.
 - 5. The Mannheimia bacteria of claim 1 wherein the Mannheimia bacteria is Mannheimia haemolytica.
- 6. The *Mannheimia* bacteria of claim 5 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.
 - 7. The *Mannheimia* bacteria of claim 5 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.
- 25 8. The *Mannheimia* bacteria of claim 5 wherein said mutation results in deletion of all or part of said gene.
 - 9. An immunogenic composition comprising the bacteria according to any one of claims 1 through 8.

10. A vaccine composition comprising the immunogenic composition according to claim 9 and a pharmaceutically acceptable carrier.

- The vaccine composition according to claim 10 further comprising anadjuvant.
 - 12. A method for producing a gram-negative bacteria mutant comprising the step of introducing a mutation in a gene represented by a nucleotide sequence set forth in any one of SEQ ID NOS: 166, 168, 170, 172, and 174 or a species homolog thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.

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- 13. A method for producing an attenuated *Mannheimia* bacteria comprising the step of introducing a mutation in a gene represented by a nucleotide
 15 sequence set forth in any one of SEQ ID NOS: 166, 168, 170, 172, and 174 or a species homolog thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.
- 14. A purified and isolated *Mannheimia* polynucleotide comprising a nucleotide sequence selected from the group consisting of nucleotide sequences set forth in SEQ ID NOS: 166, 168, 170, 172 and 174.
 - 15. A purified and isolated *Mannheimia* polynucleotide comprising a nucleotide sequence as set forth in SEQ ID NO: 166.
 - 16. A purified and isolated polynucleotide encoding a *Mannheimia* virulence gene product, or species homolog thereof, selected from the group consisting of:
 - a) the polynucleotide according to claim 14;

b) polynucleotides encoding a polypeptide encoded by the polynucleotide of (a); and

c) polynucleotides that hybridize to the complement of the polynucleotides of (a) or (b) under moderate stringency conditions.

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- 17. A purified and isolated *Mannheimia* polynucleotide encoding a polypeptide selected from the group consisting of polypeptides having amino acid sequences set forth in SEQ ID NOS: 167, 169, 171, 173, and 175.
- 10 18. The polynucleotide of claim 17 which is a DNA.
 - 19. A vector comprising the DNA of claim 18.
- 20. The vector of claim 19 that is an expression vector, wherein the DNA is operatively linked to an expression control DNA sequence.
 - 21. A host cell stably transformed or transfected with the DNA of claim 18 in a manner allowing the expression of the encoded polypeptide in said host cell.
- 20 22. A method for producing a recombinant polypeptide comprising culturing the host cell of claim 21 in a nutrient medium and isolating the encoded polypeptide from said host cell or said nutrient medium.
 - 23. A purified polypeptide produced by the method of claim 22.

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24. A purified polypeptide comprising a polypeptide selected from the group consisting of polypeptides having amino acid sequences set forth in SEQ ID NOS: 167, 169, 171, 173, and 175.

25. An antibody that is specifically reactive with the polypeptide of claim 24.

26. The antibody of claim 25 that is a monoclonal antibody.

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27. A method of using the monoclonal antibody of claim 26 for identifying a bacteria of claims 1 or 5, comprising the steps of contacting an extract of bacteria with said monoclonal antibody and detecting the absence of binding of said monoclonal antibody.

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- A method of identifying an anti-bacterial agent comprising the steps of assaying potential agents for the ability to interfere with expression or activity of gene products represented by the amino acid sequences set forth in any one of SEQ ID NOS: 167, 169, 171, 173, and 175 and identifying an agent that interferes with expression or activity of said gene products.
- 29. A method of identifying an anti-bacterial agent comprising the steps of:
- a) measuring expression or activity of a gene product as set out in any one of SEQ ID NOS: 167, 169, 171, 173, and 175;
 - b) contacting the gene product in (a) with a test compound;
 - c) measuring expression or activity of the gene product in the presence of the test compound; and
- d) identifying the test compound as an antibacterial agent when expression or activity of the gene product is decreased in the presence of the test compound as compared to expression or activity in the absence of the test compound.